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SEPTEMBER 15, 1982

INDUSTRIAL HYGIENE REPORT

TITLE

FILE NUMBER

Effectiveness of Respirators Used
During Screwworm Adult Suppression
System (SWASS) Manufacturing
Process Study Findings

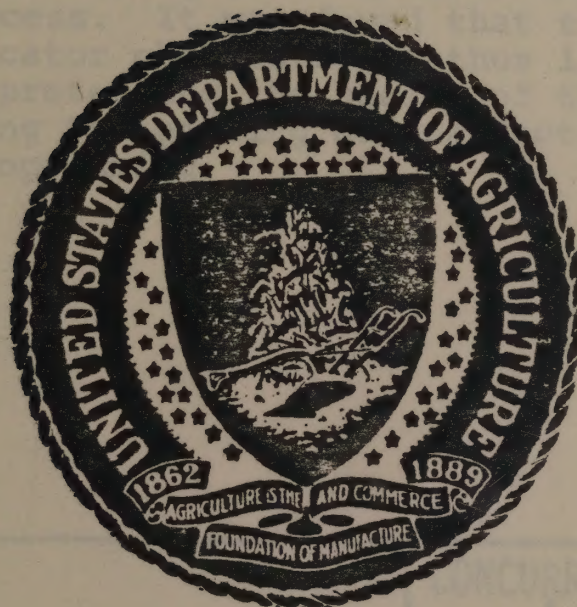
TR 82-8

INDUSTRIAL HYGIENE (15)

EFFECTIVENESS OF RESPIRATORS USED DURING
SCREWORM ADULT SUPPRESSION SYSTEM (SWASS)
MANUFACTURING PROCESS STUDY FINDINGS

ABSTRACT

A study was conducted on June 14-15, 1982 to determine the effectiveness of respirators used during the screwworm adult suppression system (SWASS) manufacturing process. It was concluded that the respirators were effective against four indicators. It was concluded that the employees, management, and the provision of short training program.



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Mr. Team Leader

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U. S. DEPARTMENT OF AGRICULTURE
WASHINGTON, D. C. 20250

UNITED STATES DEPARTMENT OF AGRICULTURE
OFFICE OF FINANCE AND MANAGEMENT
SAFETY AND HEALTH MANAGEMENT DIVISION

INDUSTRIAL HYGIENE REPORT

TITLE

Effectiveness of Respirators Used
During Screwworm Adult Suppression
System (SWASS) Manufacturing
Process Study Findings

FILE NUMBER

TR 82-8

DATE

September 15, 1982

INDUSTRIAL HYGIENIST(S)

John Teske

TYPE REPORT

Research

ABSTRACT

A study was conducted on June 14-15, 1982 to determine the effectiveness of respirators used during the Screwworm Adult Suppression System Manufacturing Process. It was found that the respirators were effective against four indicator chemicals and thus it was concluded that the respirators were protecting the health of the employees. Recommendations were made regarding the posting of instructions and the provision of short training programming.

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John W. Teske
I. H. Team Leader

DATE 9/28/82

APPROVED

Julian J. [Signature]
Division Chief

DATE 9/30/82

EFFECTIVENESS OF RESPIRATORS USED DURING SCREWORM ADULT SUPPRESSION SYSTEM MANUFACTURING PROCESS STUDY FINDINGS

TABLE OF CONTENTS

Executive Summary	1
Respirator Effectiveness	2
Protection Factors	5
Fit Testing	6
Study Protocol and Equipment	7
Results and Recommendations	9
Bibliography	12
Appendix	17

EXECUTIVE SUMMARY

Air purifying respirators are subject to question regarding their ability to protect the health of the wearer. Such a respirator may fail to provide the expected protection due to the loss or lack of facial seal, or the overloading of the filtering device or cartridge. This study was conducted on June 14-15, 1982, to determine if the respirators worn by the Screwworm Adult Suppression System (SWASS) employees were effectively protecting their health. The results of the study indicate that under the conditions of this study, the respirators were performing as expected and were protecting the wearers health.

This was determined by measuring the airborne chemical vapor concentrations inside and outside the respirator face piece using a new adaptation of existing industrial hygiene monitoring equipment. The procedures and equipment used permitted real time quantitative fit testing under conditions of actual respirator utilization. Recommendations were made, however, for the posting of written instructions in Spanish and English and the provision of training sessions for the respirator wearers.

RESPIRATOR EFFECTIVENESS

A number of factors are necessary for an air purifying respirator to be effective in protecting a wearer from hazardous or unhealthy concentrations of chemical vapors. The four major factors are that (1) the air purifying capability of the respirator must be sufficient to lower the chemical vapor concentrations inside the respirator to a healthful level (2) the respirator must provide a tight facial seal (3) the wearer must be psychologically and physiologically capable of wearing a respirator and (4) there must be an effective respirator training and maintenance program. The effectiveness of a respirator diminishes when these conditions are not met. Each of these factors will be discussed as they relate to the American Optical pesticide respirators being utilized for SWASS manufacturing during this study.

(1) Respirator Air Purifying Capability

Air purifying respirators designed for removing chemical vapors usually do so by an interaction of the vapor molecules with a sorbent material, as the air is passed through a bed of the material. The vapor molecules are removed from the passing air stream through the action of condensation, molecular attraction and electron transfer also known as chemisorption. The molecules are collected in the pores and interstitial spaces of the sorbent bed. Obviously the more porous the sorbent bed is the more efficient the chemical vapors are removed. The most common sorbent used for respirators is granular activated charcoal with millions of pores creating an internal surface area ranging from 600 to 1200 square meters/gram. The storage or sorption capacity of a charcoal bed is limited to the size (depth and volume) of the bed as well as the chemical interaction characteristics with the sorbent.

A testing and certification program for respirators is established under 30 CFR 11 which is jointly administered by the National Institute for Occupational Safety and Health (NIOSH) and the U.S. Bureau of Mines (USBM). Respirators certified under this program carry a NIOSH or Mining Safety and Health Administration (MSHA) approvals. Subpart M-Pesticide Respirators is applicable to the respirators used for SWASS manufacturing. Performance testing for certification require that the respirator be worn by a volunteer in a test chamber containing isoamyl-acetate vapor to determine facial seal leakage. Also, respirators are mounted on a mechanical-testing apparatus and exposed to silica dust, lead fume, dioctyl-phthalate, and carbon tetra-chloride to test for efficacy. The certification tests do not require the testing of respirators in the presence of pesticides. Therefore their effectiveness in protecting against specific pesticides is unknown at the time of useage.

(2) Tight Facial Seal

A tight facial seal of the respirator facepiece is essential in order that the chemical vapors do not leak around the respirator into the breathing zone of the wearer. Some factors that would prevent a tight facial seal include the selection of the wrong size respirator, unusual facial features, or excess facial hair at the facepiece seal. Also, straps that are too loose or too tight will affect facial seal.

Until recently, respirators were manufactured only in one size that was designed to fit the average male population. It was often difficult or impossible to obtain an adequate respirator fit by females and males with smaller facial sizes, by males with unusually large facial sizes, or ethnic groups with low or nonexistent nose bridges. This has been rectified to some extent by manufactures producing respirators in two to three sizes. Therefore it should be possible to obtain a correctly sized respirator that can provide a tight facial seal.

Unusual facial features that would prevent a tight facial seal include deep skin creases, scars, lack of teeth or dentures, bone deformities or deep receding chins. Any of these conditions would prohibit an individual from obtaining the maximum protection from a respirator. This would also be true of individuals that had facial hair lying between the sealing surface of the facepiece and the wearers skin. The amount of growth and the thickness of the hair would be the deciding factors on preventing a good seal. Generally, heavy stubble, sideburns, beards or moustaches that lie between the facial seal and the skin will allow leakage into the respirator.

(3) Physiological And Physiological Factors

Wearing any type of respirator places a physiological and psychological stress on the wearer. If the stress is sufficient, the wearer may not be able to effectively use a respirator. Physiological factors that would limit or prohibit wearing a respirator would be pulmonary impairment such as emphysema, chronic obstructive disease, bronchial asthma or other disease that impairs respiratory function. Individuals with a history of cardiovascular impairment should also wear respirators with caution since the additional stress of restricting air flow could worsen an existing health problem. Other physiological conditions that could limit wearing of respirators are diabetes, epilepsy, skin sensitivity or dermitites, and perforated ear drums that allow chemical vapors to pass through the eustachian tube into the respiratory system. Also, under conditions of high physical stress such as heavy lifting at high temperatures and humidity, respirators may be impossible to wear.

Psychological factors that would preclude the wearing of a respirator include claustrophobia or anxiety due to being confined to a small space. In addition, respirators that produce irritations or other discomforts will also elicit the rejection of the respirator and thus reduce its effectiveness.

(4) Respirator Training and Maintenance Program

In order that the effectiveness of respirators can be maximized, the wearers should be trained in its proper use and the respirator should be regularly maintained. Respirator training can range from a complete formal program to on the job instruction from the supervisor. Instructions should include the proper wearing and fitting of the respirator, its limitations, and how to recognize and handle malfunctions. This information and training should be given to both supervisors and employees at the time they are initially given a respirator.

Wearing a poorly maintained or malfunctioning respirator is more dangerous than not wearing a respirator at all due to the presence of a false sense of security. Inspection and maintenance should therefore be routinely be performed for defects and the respirators should be regularly cleaned. Worn or broken parts should be immediatly replaced or else the respirator taken out of service.

PROTECTION FACTORS

As a guide to the proper selection of a respirator, protection factors have been determined that the respirator should be able to meet. A respirator protection factor is the ratio of the contaminant concentrations outside and inside the respirator ($PF = \text{concentration outside} / \text{concentration inside}$). For an air purifying respirator such as being used on the SWASS program, the respirator should be able to provide a protection factor of ten. The chemical vapor concentration inside the respirator should also be low enough so as to not affect the health of the wearer. Also, the respirator should be able to provide this protection factor under all conditions of use. The protection factor therefore is a measure of the respirators ability to purify the air as well as its ability to provide a tight facial seal.

FIT TESTING

Several fit testing procedures are available to determine the adequacy of the respirator facial seal. One type of test is the positive or negative pressure test where the inlet and/or exhaust of the respirator is closed off and the wearer attempts to inhale or exhale. Leakage is usually detected by the respirators inability to maintain pressure or suction. Another procedure uses a chemical or irritant smoke or vapor that is aspirated close to the facial seal. Any leakage is usually detected via odor or irritation. All of these procedures are called qualitative fit testing in contrast to quantitative fit testing since these procedures are subjective in nature.

Quantitative fit testing procedures are more closely allied to the tests performed during this study in that chemical vapor concentrations are measured outside and inside the face piece. Traditionally quantitative fit testing is done only in laboratories under controlled conditions. The tests conducted under this study permitted quantitative fit testing under field conditions.

The SWASS Program has its own qualitative fit testing built into the production process in that dimethyl disulfide has an odor threshold at approximately 2 parts per billion (ppb). This is considerably lower than the odor threshold of the other chemicals and considerably lower than any recognized adverse health effects. It is therefore suggested that the SWASS workers be taught only the positive and/or negative qualitative fit test. Then when dimethyl disulfide is detected while wearing the respirator it is an indication that it is time to change the cartridges and check the facial seal.

STUDY PROTOCOL AND EQUIPMENT

To determine if the respirators were effectively removing the air chemical vapors and protecting the health of the SWASS workers, it was necessary to measure the vapor concentrations inside (breathing zone) and outside (challenge) the facepiece. This required some innovation and adaptation of existing air monitoring equipment. Since previous SWASS industrial hygiene surveys have focused on four air contaminants (Iso butyl alcohol, sec-butyl alcohol, dimethyl disulfide and dichlorvos) it was decided to also monitor the respirator effectiveness for these chemicals.

To do the air monitoring two dual variable orifice manifolds were used. The manifolds are designed to obtain two simultaneous collector tube samples with individually controlled flow rates. One manifold was used to monitor breathing zone concentrations and the other was used to monitor the challenge concentration. The manifolds manufactured by Gilian Instrument Company are light and compact enough to be attached to the facepiece or the shirt lapel. A sampling probe was attached in the center of the respirator facepiece at approximately the tip of the wearers nose. The probe, obtained from Dynatech Frontier Corp, is of the type used for performing laboratory quantitative fit testing. A DuPont P-4000 pump was used to draw air through the manifolds and collector tubes. The sample line consisted of 5/16" tygon tubing with a "Y" split at the pump allowing separate sample lines for each manifold. Collector tubes used were charcoal for iso-butyl alcohol and sec-butyl alcohol, chromosorb 104 for dimethyl disulfide and XAD-2 for DDVP. After sampling, the tubes were analyzed by Clayton Environmental Consultants. A copy of their laboratory results is in the appendix of this report. Respirators used during the study were American Optical 50119 facepieces with dual R-58 cartridges.

It was expected that the concentration would be lower at the

breathing zone inside the facepiece then the outside challenge concentration. The flow rates were therefore set accordingly with higher flow rates for breathing zone monitoring. Table 1 shows the various flow rates used. The manifolds were calibrated with a bubble as a complete unit with a bubble meter before monitoring and checked afterwards. The sampling was to be conducted over several consecutive work shifts. Therefore, several pumps were required should there be a malfunction in a pump. It was necessary then to calibrate each pump and manifold set to be assured of proper air flow. The hole in the facepiece was cut with an X-Acto 1/2 inch hole punch which was slightly larger than the probe. The threaded nut on the probe was tightened with a pliers to provide a tight seal.

The purpose of the study was to determine the effectiveness of the respirators as they are presently being used. Therefore no attempt was made to provide instructions to workers beyond their present knowledge of respirator useage. This resulted in one employee using a respirator with straps that had lost most of their elasticity and another arriving with a stubble growth of beard. The third employee obtained and conscientiously used a new respirator. The employees were allowed to change cartridges as they wished. Since the SWASS chemicals have a noticeably objectionable odor it is easy to identify chemical break through of the respirators and change cartridges. Some workers changed cartridges at mid-shift during heavy exposures and others used the same cartridges for the full shift for light exposures.

The monitoring covered one individual for each of seven separate tests. Table 1 is therefore divided into seven sections. The first two sample results of each test are for the breathing zone inside the respirator. The second two sample results of each test are for the challenge atmosphere immediately outside the respirator. A comparison of these two sets of sample data permit the determination of the effectiveness of the respirators and the calculation of the protection factors.

RESULTS AND RECOMMENDATIONS

The results of this study shown in Table 1 indicate that the respirators are effectively protecting the employees from iso-Butanol, sec-Butanol, Dimethyl Disulfide, and Dichlorvos under the test conditions found at the time the study was conducted. The study was a good test of the respirator effectiveness in that it was possible to determine what effect several variables had on the protection factors. For example, facial conditions from clean shaven to stubble growth of beard were present with no apparent effect on protection. It is not recommended, however, that shaving be neglected since at some point leakage will occur. Another variable that entered into the study was the capacity of the respirator cartridge for removing airborne concentrations of the SWASS chemicals. The capacity of the cartridges is not known but apparently from this study the limit was not reached. If necessary, the saturation limit of the cartridges could be the subject at a separate study. Finally, according to this study, the protection factor for the four chemicals tested did not fall below ten. However, should there be airborne concentrations of chemical vapors significantly higher than that found during this test, it is likely that chemical vapor break-through could occur due to cartridge saturation resulting in the protection factors dropping below ten.

The conditions and apparent level of respirator useage found during this study are typical of what might be found in a reasonably run respirator program. However, there are several recommendations below that should be implemented to improve the program.

1. Instructions should be posted in the rest area (English and Spanish) on the proper use and limitations of respirators as well as maintenance procedures.

2. A short training session should be available for all new-employees as well as refresher for existing employees on the proper use, limitations and maintenance of respirators. It is envisioned that one of several currently available films or slide tapes can be used for this purpose.

RESPIRATOR EFFECTIVENESS STUDY
SAMPLING RESULTS
TABLE 1

Sample Number	Type	Chemical	Sample Duration Minutes	Flow Rate l/m	Sample Vol. l.	Amt. Mg.	Conc, Mg/M ³	Prot Fact.	Remarks
<u>Test Number 1</u> - Simon Limon, Swormlure Formulation, Dayshift 6/14/82									
T-1	BZ	Iso-Butanol	68	.642	43.7	<0.002	0		Respirator Straps Det- erorated.
		Sec-Butanol	68	.642	43.7	<0.002	0		
T-2	BZ	Dimethyl Disulfide	68	.232	15.8	<0.0005	0		Same cartridges used entire Shift.
T-3	Chlng	Iso-Butanol	68	.475	32.3	0.083	2.6	>10	
		Sec-Butanol	68	.475	32.3	0.13	4.0	>10	
T-4	Chlng	Dimethyl Disulfide	68	.074	5.0	0.02	4.0	>10	
<u>Test Number 2</u> - Simon Limon, Swormlure Formulation, Dayshift 6/14/82									
T-5	BZ	Iso-Butanol	84	.632	53.0	<0.002	0		
		Sec-Butanol	84	.632	53.3	<0.002	0		
T-6	BZ	Dimethyl Disulfide	84	.230	19.3	<0.0005	0		
T-7	Chlng	Iso-Butanol	84	.475	39.9	<0.002	0		
		Sec-Butanol	84	.475	39.9	<0.002	0		
T-8	Chlng	Dimethyl Disulfide	84	.074	6.2	0.011	1.8	>10	

RESPIRATOR EFFECTIVENESS STUDY
SAMPLING RESULTS
TABLE 1 (Continued)

Sample Number	Sample Type	Chemical	Sample Duration Minutes	Flow Rate l/m	Sample Vol. l.	Amt. Mg.	Conc ₃ Mg/M	Prot Fact.	Remarks
<u>Test Number 3</u> - Apolonio Prado, Pelletizing, Evening Shift 6/14/82									
T-9	BZ	Dichlorvos	233	.634	147.7	<0.0006	0		New Respirator and Cartridges
T-10	BZ	Dimethyl Disulfide	233	.230	53.6	0.017	0.32		
T-11	Chlng	Dichlorvos	233	.475	110.7	0.099	0.89	>10	
T-12	Chlng	Dimethyl Disulfide	233	.074	17.7	0.27	15.7	49	
<u>Test Number 4</u> - Apolonio Prado, Pelletising, Evening Shift 6/14/82									
T-13	BZ	Dichlorvos	125	.634	79.3	<0.0006	0		
T-14	BZ	Dimethyl Disulfide	125	.230	28.8	<0.0005	0		
T-15	Chlng	Dichlorvos	125	.475	59.4	0.064	1.08	>10	
T-16	Chlng	Dimethyl Disulfide	125	.074	9.3	0.20	21.5	>10	

RESPIRATOR EFFECTIVENESS STUDY
SAMPLING RESULTS
TABLE 1 (continued)

Sample Number	Sample Type	Chemical	Sample Duration Minutes	Flow Rate l/m	Sample Vol. l.	Amt. Mg.	Conc ₃ Mg/M	Prot Fact	Remarks
<u>Test Number 6</u> - Apolonio Prado, Pelletizing, Evening Shift 6/15/82									
T-23	BZ	Dichlorvos	240	.634	152.2	0.0008	0.005		New Cartridges
T-24	BZ	Dimethyl Disulfide	240	.230	55.2	<0.0005	0		
T-25	Chlng	Dichlorvos	240	.475	114.0	0.015	0.13	26	
T-26	Chlng	Dimethyl Disulfide	240	.074	17.8	0.25	14.0	>10	
<u>Test Number 7</u> - Apolonio Prado, Pelletizing, Evening Shift 6/15/82									
T-27	BZ	Dichlorvos	47	.634	29.8	<0.0006	0		New Cartridges
T-28	BZ	Dimethyl Disulfide	47	.230	10.8	<0.0005	0		
T-29	Chlng	Dichlorvos	47	.475	22.3	0.0029	0.13	>10	
T-30	Chlng	Dimethyl Disulfide	47	.074	3.5	.018	51.4	>10	

RESPIRATOR EFFECTIVENESS STUDY
SAMPLING RESULTS
TABLE 1 (continued)

Sample Number	Sample Type	Chemical	Sample Duration Minutes	Flow Rate l/m	Sample Vol. l.	Amt. Mg.	Conc ₃ Mg/M ³	Prot Fact.	Remarks
<u>Test Number 5 - Francisco Rodriguez, Swormlure Formulation, Day Shift 6/15/82</u>									
T-19	BZ	Iso-Butanol	140	.634	88.8	<0.002	0		Stubble growth of beard. Same cartridges used entire shift.
		Sec-Butanol	140	.634	88.8	<0.002	0		
T-20	BZ	Dimethyl Disulfide	140	.230	32.2	<0.002	0		
T-21	Chlng	Iso-Butanol	140	.475	66.5	0.12	1.8	>10	
		Sec-Butanol	140	.475	66.5	0.19	2.9	>10	
T-22	Chlng	Dimethyl Disulfide	140	.074	10.4	0.039	3.8	>10	

This report was prepared by: **JOHN W. TESKE**

1. Feichand, John, A Guide to Industrial Respiratory Protection
 NIOSH Pub. No. 78-103 (1977)

2. Douglas, B. D. et al, Health Research and Development
Administration Division of Science, Standards and Statistics
Los Alamos Scientific Laboratory

John W. Teske

JOHN W. TESKE, CIH

Sr. Industrial Hygienist Alfred P. Johnson & Associates
 Building, 37th (1980)

4. Respiratory Protection Manual (1978) (1985)

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Respiratory Protection

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2. Douglas, D.D. et al, Energy Research and Development
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Guideline, AIHA (1980)
4. Respiratory Protective Devices Manual AIHA. (1963)
5. OSHA General Industry Safety and Health Standards, 1910.134
Respiratory Protection

Appendix

July 26, 1987

Mr. John Teske
 U.S. DEPARTMENT OF AGRICULTURE
 1401 I Independence E.W.
 GSW, Room 404
 Washington, DC 20250

ECO Lab No. 15125-022-1A
 P.O. No. 40-1100-2-1742
 Ref. No. VS 301

Dear Mr. Teske:

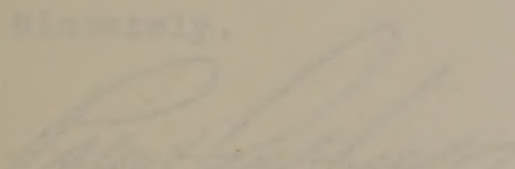
The samples which you submitted to us on June 18, 1987, have been analyzed as requested; the results are compiled in the enclosed tables.

It is a pleasure to be of assistance to you. Please contact us if you are studying any aspects of this report.

LABORATORY RESULTS FROM

CLAYTON ENVIRONMENTAL CONSULTANTS

Sincerely,


 Robert Blackfield Jr., C.I.G.
 Manager, Laboratory Services

21/22

Enclosures

Clayton Environmental Consultants, Inc.

25711 Southfield Road, Southfield, Michigan 48075, Telephone 313 424-8860

July 26, 1982

Mr. John Teske
U.S. DEPARTMENT OF AGRICULTURE
14th & Independence S.W.
OSHM, Room 48W
Washington, DC 20250

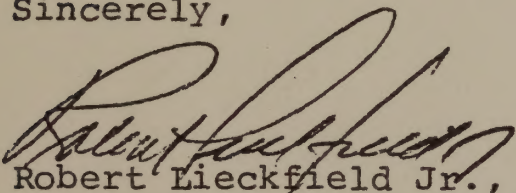
CEC Job No. 15132-682-LA
P.O. No. 40-3294-2-1748
Req. No. VS 301

Dear Mr. Teske:

The samples which you submitted to us on June 28, 1982, have been analyzed as requested; the results are compiled in the enclosed tables.

It is a pleasure to be of assistance to you. Please contact us if you have questions concerning any aspects of this report.

Sincerely,



Robert Lieckfield Jr., C.I.H.
Manager, Laboratory Services

RL/dms

Enclosures

Return

*Corpege et al
(1977)*

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ENVIRONMENTAL ENTOMOLOGY*

Field Comparisons of Liver and a New Chemical Mixture as Attractants for the Screwworm Fly^{1,2}

JAMES R. COPPEDGE,³ ELMER AHRENS,⁴ JOHN L. GOODENOUGH,³
FRANK S. GUILLOT,⁵ AND J. WENDELL SNOW³

ABSTRACT

A new mixture of the screwworm, *Cochliomyia hominivorax* (Coquerel), chemical attractant was prepared and evaluated under field conditions. This new mixture, "Swormlure-2," proved to be more attractive to screwworms than the mixture presently being used and at least as attractive as decomposing liver. In general, more unmated female flies were attracted to Swormlure-2 than were attracted to liver. Also, Swormlure-2 appeared to be more attractive than liver to unmated females that were in early stages of egg development.

An effective attractant for the adult screwworm, *Cochliomyia hominivorax* (Coquerel), that contained 10 chemical compounds was reported by Jones et al. (1976). The mixture captured 5–6 times more male screwworm flies and 7–8 times fewer other Diptera than decomposing liver, the previous attractant. However, the liver caught 2–3 times more female screwworm flies and more total screwworm flies. We improved the mixture called "Swormlure" and compared the mating and ovarian development status of the screwworm flies captured with the improved mixture (Swormlure-2) and liver. The results of these studies are reported herein.

Materials and Methods

The Bishopp type fly trap (Bishopp 1916) was used in all tests. Traps were hung ca. one m above ground level from limbs of trees. Swormlure (and Swormlure-2) was dispensed from bottles held in 33-cm diam and 20-cm deep plastic pans by circles or squares of hardware cloth (just large enough to secure the bottle upright) attached to a wooden block. When liver was used in a test, ca. one kg (previously aged 5–7 days) was placed in each bait pan, and enough water was added to cover the liver.

The initial standard mixture of the chemical attractant (Swormlure) and the general method of dispensing the attractants were described by Jones et al. (1976). Swormlure includes 3 groups of chemicals each dispensed in a single bottle, i.e., (1) acetic acid, benzoic acid, butyric acid, and valeric acid; (2) phenol and *p*-cresol; and (3) indole, isobutanol, sec-butanol, and acetone. The new mixture, "Swormlure-2," (see Table 1), was prepared by combining

the contents of bottles 1 and 3, and adding a new component, methyl disulfide, to the 2 compounds in the second bottle.

Most of the screwworm flies captured during these tests are referred to as "sterile flies." These were flies reared, sterilized, and released by Animal, and Plant Health Inspection Service, USDA, to achieve suppression of this pest. Commonly, the test areas received ca. 770 sterile flies/km² at 7-day intervals. Captured female screwworm flies suspected of being wild were dissected and the ovaries were microscopically examined for verification. The ovaries of the female screwworm flies are disintegrated by the radiation treatment. The distinction between wild and sterile male screwworm flies was made strictly on the basis of size and color.

Initially, Swormlure-2 was compared with the standard Swormlure in a field test at the Cage Ranch, in Brooks County, TX. Traps were hung 1–2 mi apart and the treatments were replicated twice. These traps were emptied at 1–2-h intervals each day, 8 h/day for 3 days. (Traps were rotated among locations daily to minimize this effect.) This test was replicated 2 times.

In the next field evaluation, Swormlure-2 was compared with decomposing liver for attractiveness to adult screwworm flies. The site for this test was the Borregos Ranch of the W. W. Jones Estate located ca. 48 km S of Hebbronville, TX. One trap baited with decomposing liver and one baited with Swormlure-2 were hung 50–100 yd apart at each of 18 sites. All sites were adjacent to windmills where livestock and screwworms were expected to congregate (Hightower and Alley 1963). Decomposing liver was placed in the bait pans on the 1st day of each test and covered with water; additional water was added as needed. Fresh attractant was added at the start of each of two 5-day testing periods.

The traps were emptied 3 times a day, and the positions of the 2 traps at each site were interchanged

¹ Diptera: Calliphoridae.

² Received for publication Sept. 13, 1976.

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⁵ Formerly with the Dept. of Entomol., Texas A&M University, presently with Agric. Res. Serv., USDA, Mission, TX 78572.

Table 1.—Composition of the new mixture of a chemical attractant; Swormlure-2.

Chemical compound	Amount per sample ^a
Bottle 1	
acetic acid (glacial)	6.25
benzoic acid	1.25 g
butyric acid	6.25
valeric acid	6.25
indole	1.25 g
isobutanol	6.25
sec-butanol	6.25
acetone	6.25
Bottle 2	
Phenol	5.00
p-cresol	5.00
methyl disulfide	5.00

^a Given in milliliters unless otherwise noted.

each time traps were emptied. The catches were transported to a mobile laboratory on the ranch where each was sorted, the flies sexed, and the numbers recorded. Female screwworms from each trap were dissected, and their spermatheca were microscopically examined to determine whether they had previously mated. Also, samples (20–25 ♀/treatment/day) of live wild female flies were daily transported to the main laboratory where they were dissected to determine the stage of egg maturation, parity and mating status. The system used in scoring the maturation stage was similar to the one described by Adams and Hintz (1969).

Results and Discussion

In the 1st field comparison (Cage Ranch Test), Swormlure-2 proved more attractive than Swormlure to adult screwworms. In this test, it captured over twice as many (539 for Swormlure-2 compared with 259 for Swormlure) screwworm flies. These data indicated that Swormlure-2 was promising enough to justify an intensive field comparison of it and liver.

In the final field comparison (Table 2), Swormlure-2 captured significantly more sterile male and sterile female screwworm flies than liver. Likewise,

traps baited with Swormlure-2 captured significantly more wild male screwworm flies than did the liver-baited traps and ca. the same number of wild females. Thus, the traps baited with Swormlure-2 captured more total wild screwworm flies than traps baited with liver; but this was not significantly different according to the statistical analysis used (Sign test, Snedecor and Cochran 1973).

A significantly higher percentage of the wild female flies attracted to Swormlure-2 and liver were mated (an average of 80 and 91%, respectively) than were the sterile female flies (an average of 47 and 62%, respectively). Also, significantly more of the sterile female flies captured with liver (average of 62%) were mated than the sterile females captured with Swormlure-2 (average of 47%). On most sampling dates, a higher percentage of the wild females captured with liver was mated than the wild flies captured with Swormlure-2; however, the average percentage of such females captured over the entire test was not significantly different.

Swormlure-2 appeared more attractive than liver to very young (nulliparous stage 3+4) females which were unmated (Table 3). Otherwise the two baits appeared quite similar in attractiveness to the nulliparous and parous females in the various stages of egg development. About 75% of the female flies attracted to liver and Swormlure-2 had developing eggs in stages 3–6. Very few of the females attracted to these baits were ready to oviposit (Stages 9 + 10).

Swormlure-2 is more attractive than liver to 2 segments of the screwworm fly population, i.e., male flies and young virgin females. The attractiveness of Swormlure-2 to virgin females may result in the typically lower percentage of mated females in the traps baited with it than in the traps baited with liver. Newly emerged sterile flies are routinely released every 7 days in the test areas in support of the Screwworm Eradication Program, consequently, the number of young unmated females in the sterile fly population is probably greater than the number in the wild fly population. The younger sterile fly population is probably the reason the chemical attractant is more attractive than liver to the sterile flies and only equally as attractive to wild flies.

In conclusion, the new mixture of the chemical attractant, Swormlure-2, offers all the advantages

Table 2.—Field Comparison of Swormlure-2 and Decomposing Liver: Jones Ranch, 1975.

Bait	No. of screwworm flies captured			Mean no. of screwworm ^a flies/day/trap		
	♂	♀	Total	♂	♀	Total
Sterile Flies						
Swormlure-2	332	1399	1731	1.8 a	7.8 a	9.6 a
Liver	39	1028	1067	0.2 b	5.7 b	5.9 b
Wild Flies						
Swormlure-2	185	963	1148	1.0 a	5.4 a	6.4 a
Liver	17	785	802	0.1 b	4.4 a	4.5 a

^a Means followed by the same letter are not significantly different at the 5% level: Sign test (Snedecor and Cochran 1973).

Table 3.—A comparison of stages of egg development in samples of wild female screwworm flies attracted to liver and Swormlure-2.

Stages of egg development ^a	Number or percent of wild female flies examined					
	Swormlure-2			Liver		
	No.	Percent of sample	Percent mated	No.	Percent of sample	Percent mated
<i>Nulliparous females</i>						
3+ 4	33	32.7	12.1	23	26.4	34.8
5+ 6	39	38.5	79.4	43	49.5	72.1
7+ 8	14	13.9	100	11	12.6	90.9
9+10	15	14.9	100	10	11.5	100
Total	101		63.3	87		67.8
<i>Parous females</i>						
3+ 4	44	32.1	100	72	44.2	100
5+ 6	62	45.3	100	65	39.9	100
7+ 8	24	17.5	100	23	14.1	100
9+10	7	5.1	100	3	1.8	100
Total	137		100	163		100

^a The system used in scoring the maturation stage was a slight modification of the one described by Adams and Hintz (1969).

cited by Jones et al. (1976) for Swormlure plus the additional increased attractiveness to females. This mixture has now successfully replaced both liver and Swormlure as the attractant for the Screwworm Eradication Program.

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ENTOMOLOGIA MEDICA Y VETERINARIA

Asociación entre las masas de polen de la hierba lechosa y los adultos del gusano barrenador del ganado, *Cochliomyia hominivorax* (Coquerel)—J. W. Mackley, SEA, AR, Lab. Investig. Gusano Barrenador, Tuxtla Gutiérrez, Chis.

La polinización en Asclepiadacea o familia de las hierbas lechosas se ve afectada por la transferencia de masas de polen entre sus flores por insectos. El insecto las remueve de las flores a las que acude a alimentarse y las inserta en las flores siguientes al buscar los nectarios. Esas masas normalmente se quedan en sus uñas, pulvilos o setas de las patas y pelos del vientre. Los polinizadores más comunes de las asclepicidaceas son las abejas o abejorros.

Durante los veranos de 1979 y 1980 se recolectaron adultos esterilizadores de *C. hominivorax* Coquerel en trampas matamoscas localizadas cerca de la planta de cría del gusano barrenador del ganado, de la Comisión México-Americana en Tuxtla Gutiérrez, Chis. Masas de polen de *Genobolus barbatus* HBK (Asclepiadacea), una enredadera herbácea, se encontraron en el 0.9% de las moscas capturadas. Un pequeño porcentaje de otras moscas relacionadas (*C. macellaria* y *Phaenicia* spp.) también tenía polinias. El porcentaje de machos de *C. hominivorax* con polinias fue cerca de la mitad del de hembras (0.51 y 1.06 respectivamente). La proporción de sexos entre adultos con y sin polinias fue similar (las hembras representaban al 81 y 72% respectivamente).

Sin excepción se encontraron polinias en la proboscis, generalmente cerca de la base del rostrum. Casi todas las polinias estaban intactas por lo que pocas habían sido insertadas en las flores. El promedio de polinias por adulto fue de 5.02 ± 4.26 ($n = 245$ y con un rango de 1-20 polinias). El 57.7% de las hembras con polinias ya habían copulado y sólo el 16.3 de las que no tenían, lo había hecho.

La aparición de polinias en las partes bucales es rara o accidental (Robertson, 1887 Macio, 1965).

Sin embargo se considera poco probable que estos adultos y otros rela-

cionados sean polinizadores importantes de *G. Barbatus* HBK. De hecho interfieren la polinización al reducir el nectar y el polen que atraen a los polinizadores. Como las hembras ya copuladas y con polinias eran las más abundantes se infiere que *G. barbatus* sirve como lugar de cópula. La abundancia de polinias puede afectar la supervivencia adulta al interferir su alimentación y su estabilidad durante el vuelo.

Factores que determinan el establecimiento de poblaciones larvales de *Simulium ochraceum* (Walker) (Diptera: Simuliidae) en el declive pacífico de la Sierra Madre de Chiapas—Julio Montoya Palacios y Mauricio Ortega G., Centro de Investigaciones Ecológicas del Sureste.

Siendo *Simulium ochraceum* el principal vector de la oncocercosis en México y Guatemala, es importante caracterizar a nivel preciso su microhabitat para tener mayores oportunidades de éxito en su control.

El presente estudio tiene como objetivo determinar qué factores tanto bióticos como abióticos y en qué magnitud, son los responsables de la distribución y densidad de poblaciones larvales de *S. ochraceum*.

El estudio se efectuó en cuatro comunidades de la Sierra Madre de Chiapas y durante la época lluviosa del presente año. Se abordaron los siguientes aspectos:

1. Establecimiento global de las condiciones ecológicas del área de estudio, topografía, clima, vegetación, etc.
2. Estudio intensivo de los factores propios de las corrientes (criaderos).
 - 2.1 Factores físicos: anchura, profundidad, velocidad de corriente, etc.
 - 2.2 Factores químicos: pH, alcalinidad, dureza, etc.
 - 2.3 Factores bióticos: cantidad y tipo de sustratos, vegetación bordeante, etcétera.

Los resultados nos hacen ver que a nivel microhabitat, los factores más importantes para el establecimiento de esta especie, son en primer lugar, la cantidad y tipo de sustrato disponible para la fijación de las larvas, y en segundo lugar, las características físicas de la corriente, como volumen y velocidad.

Se han obtenido las siguientes conclusiones:

1. El tipo de sustrato ideal son las hojas anchas de amplia exposición

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Our Ref PDC/TG

20th June 1983

The Director
Sterile Screw Fly
Production Plant
Mission
Texas
United States

*Answer letter &
Suggest Trip To Mex. Program -
and Fly Plant*

Dear Sir,

In February 1979 we had the opportunity to visit two of the U.S.D.A research establishments for entomological studies, one at Gainesville in Florida, and the other at Mission in Texas.

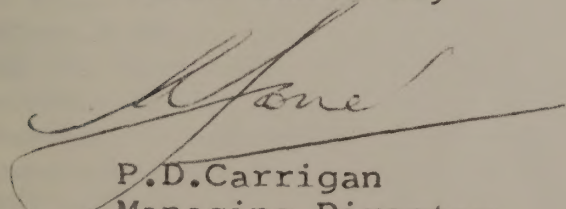
The latter was of specific interest to us as it deals with the "Screw Worm Fly". Our interest is similar as we breed maggots on a large commercial scale for the fishing industry. The institute was at that time Directed by Mr J. Wendel Snow, and we spent much time with his very able team Dr Harold Brown and Mr Lloyd Wendell.

We would once again very much like to visit the U.S.A to further our knowledge and indeed offer reciprocal visits to any U.S.D.A. staff who may wish to visit us in England.

Our particular interest is research into any flesh eating insects particularly those associated with dead animals or carrion.

Perhaps you would be so kind as to investigate the U.S.D.A research centres likely to be of interest to us, and accept this letter as our request to visit. It is our intention, subject to your approval to come to America in the late Autumn of this year.

Yours sincerely


P.D. Carrigan
Managing Director

Dictated by Mr P.D. Carrigan
and signed in his absence

SCREWORM FLIES (DIPTERA: CALLIPHORIDAE): INFLUENCE OF SEX RATIO ON PRIMIPAROUS FERTILITY OF CAGED POPULATIONS

Abstract: The primiparous fertility of caged screwworm flies, *Cochliomyia hominivorax*, was similar whether the ratios of males to females were 2:1, 1:1, or 1:2. Fivefold sex ratios had adverse effects on fertility except when an excess of females was accompanied by an increase in cage space per fly.

In laboratory experiments with screwworms, *Cochliomyia hominivorax* (Coquerel), maximum numbers of matings and progeny must often be produced. However, in some cage populations in which sex ratios departed from unity, fertility of the first egg masses laid (i.e., the primiparous fertility) seemed to be less than expected. Tests were therefore conducted to determine more precisely the effects of different sex ratios.

All tests were conducted with mass-reared screwworm flies (>100 million/week) of a strain originally developed in Texas in 1973 from 25 collections of natural infestations (Crystal & Whitten, 1976, Ann. Entomol. Soc. Am. 69: 621-24). The test insects were obtained as pupae from the screwworm fly plant where immature stages are held in total light (24 h), and adults in total darkness (24 h). The pupae and emerged adults were held in the laboratory at 27°C and 60% RH with a 12-h photophase beginning at

0600 h. Groups of 60 unmated 5-day-old flies consisting of male: female ratios 1:5, 1:2, 1:1, 2:1, and 5:1 were placed in separate cages ("standard"), each 12.5 × 12.5 × 30.0 cm (ca 0.005 m³ capacity), that were covered with seamless tubular cotton gauze fabric. Ten similar cages were set up with 10 females plus the appropriate number of males to produce the same ratios; however, the lengths (6.0-30.0 cm) of 5 of these cages ("proportional") were such that the cage space per fly was the same for all ratios. Eggs were obtained 2 days after the cages were set up by confining all females individually for 2-4 h at 37°C in 30-ml shell vials containing ground meat. The hatchability of eggs from inseminated females (verified by dissection of ovipositing females) was determined. Each test was repeated independently on 4 dates, and the results were averaged for tabulation. Differences were tested by calculating chi-square.

Survival, oviposition, and fecundity were similar in all cages irrespective of sex ratio, and these data are not presented here. However, percentage hatch was affected by sex ratio, and the data for egg hatch are given in TABLE 1. In cages affording equal space per fly (columns 2 and 4), hatch was greatest when there were as many or twice as

TABLE 1. Percentage hatch of eggs laid by screwworm ♀♀ placed with ♂♂ in different sex ratios (avg. of 4 replications).

SEX RATIO (♂:♀)	% HATCH* OF INDICATED POPULATION**		
	60 flies/standard cage***	10 ♀♀/standard cage***	10 ♀♀/proportional cage***
1:5	62.6 a x	82.4 a y	66.3 a x
1:2	71.9 b x	77.2 ab xy	81.0 b y
1:1	76.5 b x	75.9 b x	76.1 b x
2:1	74.7 b x	66.2 c y	78.9 b x
5:1	54.2 c x	62.9 c y	69.4 a z

*Each ovipositing ♀ laid 220 (avg.) eggs that yielded 900–8900 eggs/cage. Hatchability figures based on 546 (avg.) eggs examined for each cage and replication (>2000 eggs total).

**Observations within a column (a,b,c) or row (x,y,z) that share the same letter are not significantly different from one another at the 5% level.

***Standard cage: 12.5 × 12.5 × 30.0 cm; proportional cage: 12.5 × 12.5 × 6.0–30.0 cm.

many of one sex as the other. In cages affording progressively reduced space per fly (column 3), hatch was greatest at the lowest ratio of males to females, and it decreased progressively as the ratio was increased, though the hatch of most vertically adjacent pairs did not differ significantly.

The data show that the hatch of eggs from the test insects was influenced by the sex ratio. In previous tests, hatch was reduced when females outnumbered males at least 10 times (Baumhover, 1965, J. Econ. Entomol. 58:

544–48; Crystal, 1967, J. Med. Entomol. 4: 443–50). In the present tests, a 5-fold excess of either sex led to reduced hatch in populations of equal density. Also, at the 1 male: 5 female ratio, hatch was further modified by the density of the males. When 10 females were present, hatch was greater (82.4%) at a low density of males (2/0.005 m³) than at a higher density (66.3% at 2/0.001 m³), probably because competition for mates among males was greater at the higher density than at the lower. Thus, a male at the lower density would have been likely to mate more frequently and to complete copulation and sperm transfer more often without interruption by competing males. In the 3 tests of the 5 male: 1 female ratio, the populations were identical in size, and no explanation for the differences in the results is readily apparent. Nevertheless, the hatch for populations with twice as many of one sex as the other was similar to the hatch for populations with equal numbers of both sexes. Also, a 5-fold sex ratio influenced hatch adversely except when an excess of females was accompanied by an increase in cage space per fly.

I thank Roberto Ramire of this laboratory for his help in conducting these experiments. —**Maxwell M. Crystal**, Screwworm Research Laboratory, Agric. Res. Serv., USDA, P. O. Box 986, Mission, Texas 78572, USA. Present address: Biological Evaluation of Chemicals Laboratory, Agricultural Environmental Quality Institute, Agric. Res. Serv., USDA, Beltsville, Maryland 20705, USA.

Adult Screwworms¹: Comparison of Captures in Wind Oriented and Electrocuter Grid Traps²

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ABSTRACT

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Captures of male and female screwworm, *Cochliomyia hominivorax* (Coquerel), secondary screwworm, *C. macellaria* (F.), and other flies (mainly sarcophagid) were 1.7, 1.7, 6.3, and 6.2 times greater, respectively, in an electrocuter grid trap than in the newly developed wind oriented trap (WO). Higher captures by the grid trap than the WO traps in both types of habitats (windy and protected) indicate that design modifications of the WO trap are necessary for it to achieve its full potential of performance.

Goodenough and Snow (1973) and Hollingsworth et al. (1977) demonstrated the superiority of electric grids baited with pheromone over a variety of nonelectric designs for capturing night-flying insects. For example, when a battery-powered electrocuter grid trap was used to capture the day-flying screwworm fly, *Cochliomyia hominivorax* (Coquerel), Goodenough and Snow (1977) found that the grid trap captured 92 times more male screwworms, 56 times more female screwworms, and 11 times more secondary screwworms, *C. macellaria* (F.), than the conventional screen trap as developed by Bishopp (1916) and modified by A. H. Baumhover (unpublished data) when both were baited with a chemical attractant (Coppedge et al. 1977). The large differences in captures pointed to the ineffectiveness of the screen trap and prompted Broce et al. (1977) to develop a wind oriented (WO) trap for capturing screwworm flies. The WO trap was up to 6 times more effective than the screen trap in capturing screwworm flies (ratios of geometric means from 10 traps; 2 day-captures), cheaper to build, and easier to service. It caught only ½ as many trash flies (mainly secondary screwworm flies) as the screen trap which further enhanced its value because considerable time and expense are required to identify and separate captured secondary screwworm from screwworm. For these reasons, the WO trap was used almost exclusively during 1977 for survey and detection of screwworm flies by the USDA—Animal and Plant Health Inspection Service (APHIS) in Texas and Mexico, and by the USDA—SEA in the large scale test of Screwworm Adult Suppression System (SWASS) units in Curacao (Coppedge et al. 1978). To determine the relative effectiveness of the electrocuter grid and WO traps, I compared captures of screwworms, secondary screwworms, and other flies in these traps on the island of Curacao, Netherlands Antilles.

Materials and Methods

The grid trap (Fig. 1) had a cylindrical grid element identical to that used by Mitchell et al. (1972): it was 46 cm high and 30 cm diam with a nominal electrode spacing of 0.64 cm between positive and negative electrodes. In this trap, the chemical attractant (swormlure-2, Coppedge et al. 1977) was centrally located inside the grid element in 2 bottles. Each bottle contained ca. 30 ml of

chemical, and No. 2 cotton dental wicks protruded ca. 1.5 cm from each bottle to dispense the chemical. Attracted flies struck the grid, were knocked down via the ca. 2000-V AC delivered across the grid elements, and dropped into the collection container, a plastic pail like that used in the WO trap (Broce et al. 1977). The grid trap was mounted on a portable wooden stand so that it could be moved between the 2 sites. The center of the grid element was ca. 1.5 m above the ground.

The wind oriented trap (Fig. 2) was identical to the trap described by Broce et al. (1977). (Construction details were included in their report.) This trap catches flies as they fly upwind toward the attractant. The same chemical, containers, etc., were used as for the grid trap. They were placed in the wire holder shown at the left end of the trap in Fig. 2. In use, the trap was suspended ca. 1.5 m above the ground by swivel and eye hooks that offered low resistance to wind orientation.

The 2 sites where the traps were exposed were distinctly different: the 1st site (designated as the protected site, P, Fig. 1 and 2) was characterized by thick growth of trees and bushes. Traps located at this site were protected from wind. The other site (designated as the windy site, W, Fig. 3) was a ranch ca. 6.5 km to the east. There, although the traps were placed beneath a tree, undergrowth was sparse so the traps were open to the prevailing winds which blew from the SE at ca. 13–24 km/h.

Collections were made daily from July 21 until Aug. 9, 1977, for a total of 20 replications. Traps were interchanged between the 2 locations after each collection. Care was taken to locate both types of traps at the same height (ca. 1.5 m) at both sites. All capture data were transformed to log (x + 1) before analysis. (Comparison of geometric means was proposed by Williams (1951) as the most appropriate transformation to normalize insect capture data.) Paired *t*-tests (*P* < 0.05) or analysis of variance tests were made to compare trap captures. Two-way analysis of variance tests were calculated to compare captures between the 2 trap sites.

Results and Discussion

The grid trap caught significantly more male screwworms, secondary screwworms, and other flies than the WO trap (Table 1). Ratios of geometric mean captures of WO: grid showed that 1.7 times more male and female screwworms, 6.3 times more secondary

¹ Diptera: Calliphoridae.

² Received for publication Oct. 11, 1978.

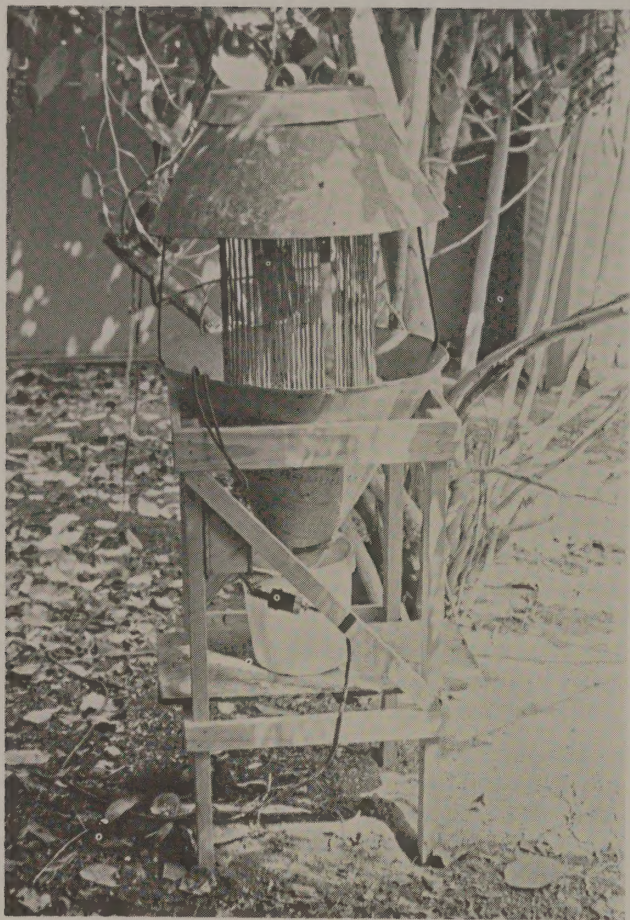


FIG. 1.—Electrocutor grid trap at protected site.



FIG. 2.—Wind oriented trap at protected site.

screwworms, and 6.2 times more other flies (mainly sarcophagid) were captured in the grid trap than in the WO trap. The majority, if not all, of the screwworm flies captured was sterile flies being released to eradicate the native screwworm population on the island (Coppedge et al. 1978).

The grid trap caught more flies of each species at both sites (Table 2) but the difference was not significant for male screwworm at site W or for female screwworm at either site. The significant difference between grid-trap and WO-trap captures of male screwworm noted before apparently resulted from the difference in male screwworm captures at site P. The relative difference between grid-trap and WO-trap captures appeared ca.



FIG. 3.—Windy site with electrocutor grid trap.

Table 1.—Screwworm fly captures in an electrocutor grid trap and a wind oriented (WO) trap.

Species	Mean capture/trap/day ^a			
	WO trap	Grid trap	Ratio screen: grid	Significance level ^b
Screwworm:				
males	1.12a	1.95b	1:1.7	*
females	2.19a	3.63a	1:1.7	NS
Secondary screwworm (♂ + ♀)	1.26a	7.94b	1:6.3	**
Other flies	1.07a	6.61b	1:6.2	**

^a Geometric means obtained by using log (x_i + 1). Means in rows not followed by the same letter are significantly different, *P* < 0.05, Duncan's multiple range test. All values are for captures at both sites.
^b NS indicates means not significantly different, *P* < 0.05; *, **, indicate means significantly different at *P* < 0.05, *P* < 0.01, respectively.

captures make it plain that the WO trap can be further improved for survey and detection of screwworm flies. In fact, unpublished data of J. R. Coppedge and separate unpublished data of A. B. Broce, both of this laboratory, indicate that ca. 1/2 of the screwworm flies hand-placed in a WO trap escaped within 24 h. Therefore, the measured difference in the grid and WO captures may actually result from loss of flies from the WO trap after capture. Another possibility, though not observed by Broce et al. (1977), is that some flies attracted to the WO trap do not enter.

If physical modifications do not improve WO-trap capture, the electric grid is an alternative. However, this would be more costly (\$50–200 for the grid trap compared with \$6.50–13 for the WO trap), and modifications to the grid trap would have to be made to reduce the numbers of nonscrewworm flies captured. It is possible that behavioral differences in the species reported by Broce et al. (1977) could be exploited. They reported

Table 2.—Captures in an electrocutor grid trap and a wind oriented (WO) trap at 2 types of habitat.

Species	Mean capture/trap/day ^a					
	Windy site (W)			Protected site (P)		
	Grid trap	WO trap	Grid vs. WO ^b	Grid trap	WO trap	Grid vs. WO ^b
Screwworm:						
males	1.90	1.26	NS	1.98	0.0	*
females	5.93	3.96	NS	2.22	1.20	NS
Secondary screwworm (♂ + ♀)	31.0	3.96	**	2.02	0.0	*
Other flies	14.4	1.15	**	2.99	0.0	*

^a Geometric means using log (x_i + 1).
^b Results of analysis of variance tests on the data transformed by log (x_i + 1); NS indicates means not significantly different, *P* < 0.05; *, **, indicates means significantly different at *P* < 0.05 and *P* < 0.01, respectively.

consistent between sites even though there were large differences in mean captures. Thus, lower captures in the WO trap appeared to have been a characteristic of the trap that was generally consistent at both sites and not a reflection only of population differences between sites. Total captures were significantly higher at site W than at site P for all categories except male screwworm (comparison based on combined data (not shown) from both traps—analysis of variance test, *P* < 0.01). The relatively large number of secondary screwworms and other flies collected by the grid trap is a definite disadvantage because of the increased sorting time required (more discussion later).

Very low wind speed may have resulted in poor wind orientation of the WO trap at site P. This lack of trap orientation may have contributed to lower captures of this trap, but it is not likely that the relatively lower captures were caused only by lack of wind orientation or insufficient chemical dispersion. Reduced captures with decreasing wind speed is contrary to the prediction of chemical dispersion models that area of trap influence will increase with decreasing wind speed (Wright 1958, Hartstack et al. 1976).

The differences between the grid-trap and WO-trap

that *C. macellaria* hovered by the entrance to the WO trap, seemingly repulsed by the chemical attractant when it was close to it; *C. hominivorax* went directly into the trap. Also, changes in grid size or location of the attractant might reduce the numbers of these unwanted species in the catch. Available power is not a limitation because portable, battery-powered grid traps have previously been used successfully in trapping screwworm flies (Goodenough and Snow 1977), and solar power for keeping batteries charged has been demonstrated as technically feasible (Witz et al. 1977). It would be necessary to use a portable trap if grid traps were used on a wide scale for trapping screwworm flies.

Acknowledgment

I thank Harold Tannahill (Texas A&M Cooperator), USDA, SEA, FR Biological Technicians Alfred Siebenaler and Robert Ramirez, and the Veterinarian Services Department, Curacao, Netherlands Antilles, for their assistance.

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CAPTURE OF SCREWORM AND SECONDARY SCREWORM FLIES (DIPTERA: CALLIPHORIDAE) IN A TIME-INTERVAL GRID TRAP AND CORRELATION WITH TEMPERATURE AND HUMIDITY¹

By John L. Goodenough and J. Wendell Snow²

Abstract. A time-interval electrocutor grid trap was successfully modified to collect adult screwworms, *Cochliomyia hominivorax*, and secondary screwworms, *C. macellaria*, during 14 hourly diurnal periods. Peak captures of screwworms were made in the morning, but collections of secondary screwworms were made in larger and more uniform numbers throughout the day. The total numbers captured and the activity patterns were highly variable day to day. Seasonally, captures peaked around midday during cooler weather, but were more evenly distributed throughout the day during hotter weather. Hourly captures of both species were positively correlated with increasing temperature in the range 20° to 33 °C. Screwworm capture was very low at temperatures below 20 °C, peaked at ca 33 °C, and appeared to decrease when the temperature rose from 33° to 35 °C. Secondary screwworms were detected over a greater temperature range than were screwworms, possibly because secondary screwworms were present at much higher numbers. Captures of secondary screwworms did not decrease between 33° and 35 °C. Additionally, a high degree of variation occurred both in diurnal pattern of fly activity and in numbers captured of both species even between successive days having apparently identical temperature and humidity patterns. Relative humidity did not appear to influence captures, except that captures were zero or extremely low during rains.

Information on daily patterns of flight and relationships to temperature and relative humidity was needed to more fully understand the behavior of the screwworm, *Cochliomyia hominivorax* (Coquerel). We also studied the responses of the closely related secondary screwworm, *C. macellaria* (F.), for comparative purposes. These data will provide insight into the population dynamics of the screwworm and assist the SEA/AR and the Animal & Plant Health Inspection Service (the agency in charge of the mass rearing-release program) in the research, development, evaluation, and implementation of new release strategies.

MATERIALS AND METHODS

In the time-interval trap (FIG. 1) flies were attracted to decaying beef liver held in a 33 × 18 cm

bait pan surrounded by an electrocuting grid [Goodenough & Snow (1977) found that the electric grid was more efficient in collecting screwworms and secondary screwworms than a nonelectric trap; their trap did not include the modifications described below]. Flies attracted to the liver bait struck the electric grid and were either killed, injured, or stunned and fell into the funnel. The funnel collected and guided the captures into small screened cylinders. An inverted cone within each cylinder (FIG. 2) restricted the escape of live insects. On windy days some electrocuted flies were blown far enough to miss the funnel until plywood sheets (ca 1.2 × 1.2 m) were placed ca 60 cm from the grid as windbreaks to correct this problem.

The electric grid was a modification of the type developed by Kishaba et al. (1970). We jury-rigged it by connecting grids from 3 Detjen Corporation® Insectocutor traps electrically in parallel, and physically arranged in a triangular pattern. The grid voltage was ca 2500 V with grid spacing of ca 1 cm. To standardize bait performance at different ambient temperatures, we heated the liver bait to ca 32 °C with a thermostatically controlled Blue-M® immersion heater. This was important in cool weather because the decomposition rate (and thus odorous gas release and attractancy) of liver was dependent on temperature.

The time-interval portion of the trap was a modification of the type developed by Smith et al. (1973). We modified their device to accommodate 15 collection containers instead of 12. This required revamping the turntable to accommodate 15 containers and machining a new aluminum cam wheel (used to switch off the turntable motor) with 15 equally spaced cams (slots). (Plywood cams provided acceptable temporary substitutes.) We used only hourly periods, but the length of each capture interval was adjustable in 15-min intervals. A wiring schematic diagram for our capture chamber advance system is shown in FIG. 3. Timed operation was obtained by moving the Double-Throw

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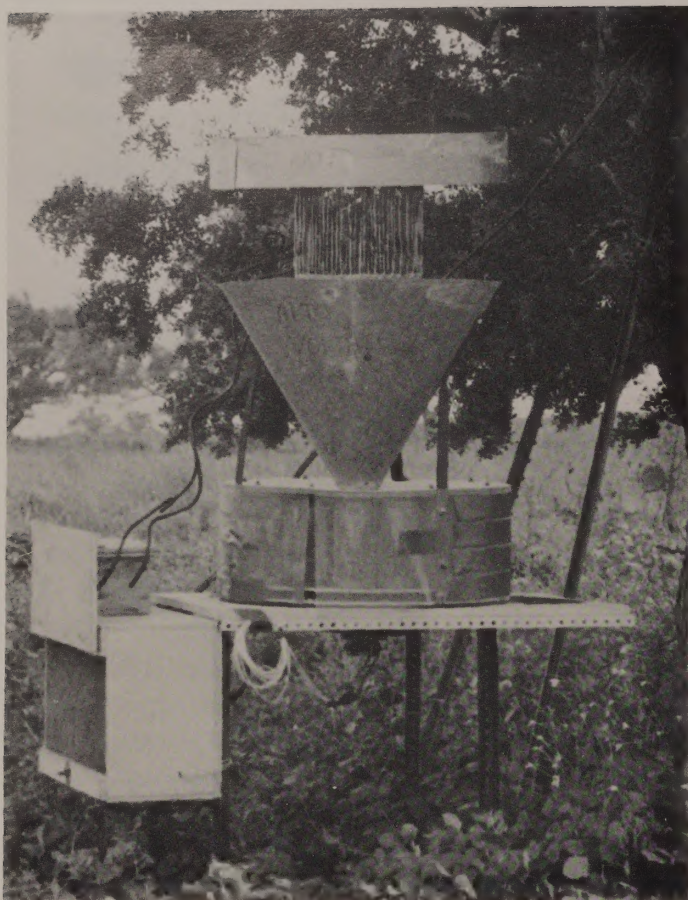


FIG. 1. Time-interval electric grid trap.

(DPDT) switch (SW) to auto position. For manual advance of the turntable, DPDT SW was moved to manual position.

Captures of screwworms and secondary screwworms were made during October and November 1974 and February and March 1975 in Kenedy County, Texas and from June to November 1975 in southern Hidalgo County, Texas. The entire screwworm capture and a portion of the secondary screwworm capture were sexed. A portion of the screwworm and secondary screwworm females captured from 10 to 28 Oct. 1974 was checked for mating by microscopic examination of spermatheca squashes. Screwworm flies were identified as native or mass-reared by size and color, a highly subjective procedure that is prone to error. However, too few native flies were captured to be included separately herein.

Collections were usually made daily except on weekends. This provided valid hourly capture data Tuesday through Thursday. Data included in the activity, temperature, and humidity relationships were those obtained for June to November 1975. Data for days of incomplete daily captures (e.g., data not available every hour throughout the day),

trap malfunction, zero daily catch of a particular species, etc., were not used. Of a possible total of 158 trapping days, usable screwworm data were available for 38 days and secondary screwworm data for 63 days. The number of trapping days per month from June to November 1975, respectively, was as follows: for screwworms 7, 9, 12, 5, 2, and 3; and for secondary screwworms 7, 12, 12, 11, 16, and 5. Capture data were coded to $\log(x_i + 1)$ for activity and to $\sqrt{x_i}$ for correlation with temperature and relative humidity to normalize the data. (The choice of $\log$ or $\sqrt{}$ was at our convenience—it made little difference in the curves).

Air temperature (T) ($^{\circ}\text{C}$) and RH were monitored with a Belfort[®] hygrothermograph operated ca 3 m from the interval trap. Mean hourly air temperature and RH were correlated with hourly captures (x_i) of screwworms and secondary screwworms. Because values of temperature and RH occurred at unequal frequencies, a mean capture per occurrence (w_i) was calculated for each 1°C and 1% RH (i.e., $w_i = \sum_{j=1}^{n_i} \{\sqrt{x_i}/n\}$). Finally, the percentage per temperature class was determined ($\%_i = \{w_i/\sum w_i\} \cdot 100$). For RH, the same procedure was used for each 1% RH class.

Best-fit polynomial equations relating activity and temperature or RH were determined by a stepwise regression procedure. The order of equations included herein is that in which the next term (next higher power of x) added did not significantly increase the coefficient of determination (R^2) of the equation (t -test, $P < 0.05$).

An additional temperature comparison was made by finding percentage/class as described above, but for each hourly capture period separately (i.e., we constructed activity vs temperature correlations for captures from 0800 to 0900 h and then separately for 0900–1000 h, etc). This was done to try to adjust for the fact that all temperatures did not occur an equal number of times at each hour of the day. This covariance procedure involved a considerable amount of work but did not increase the correlation of capture and temperature or capture and RH. We mention it here so that others may avoid this fruitless effort.

Lastly, we determined estimates of variability in captures among days of equal (to the nearest $^{\circ}\text{C}$) mean temperature. Mean daily temperatures were calculated from mean hourly temperatures (those used in the correlations with hourly captures) from 0700 to 2100 h daily. Standard deviations of captures were calculated from hourly catches of both

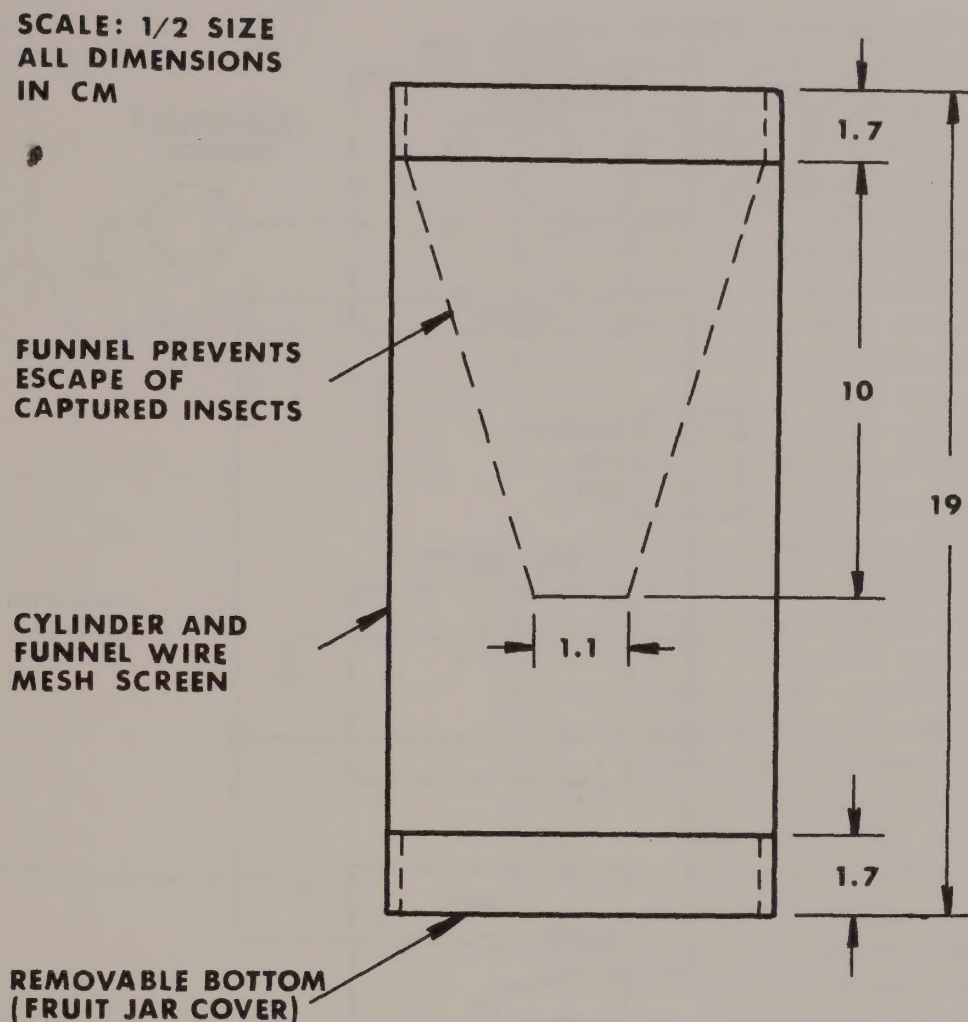


FIG. 2. Line drawing of a modified collection container (15 required).

species on these days. Hourly captures were coded to $\log(x_i + 1)$ for these determinations.

RESULTS

Mean activity and estimated variability of activity of mass-reared female screwworm and secondary screwworm (both sexes combined) flies are shown in FIG. 4. The smooth curves are hand drawn. Vertical bars denote ca 95% confidence intervals (a binomial distribution was assumed). Both species were caught throughout the day, but secondary screwworms were caught in much higher numbers. Screwworm captures were highest in the morning, with a possible secondary peak in the afternoon. Screwworm captures from 0900 to 1000 h and 1100 to 1200 h were significantly higher (t -test, $P < 0.05$) than at 1200 to 1300 h, but not higher than any others except those from 0700 to 0900 h or after 1800 h. Norris (1966) reported pronounced morning and afternoon peaks in trap catches of *Calliphora stygia* (F.) and *C. augur* (F.) in hot weath-

er, but unimodal curves of trap catches in colder months. We suspect similar trends, but captures were too low in winter to determine if curves were truly unimodal. It was unfortunate that native female screwworms were captured in numbers too limited for comparison of their activity with that of recaptured sterilized mass-reared females. There was little difference in secondary screwworm activity from 0900 to 1900 h. Captures before 0900 h and after 2000 h were significantly lower than between 0900 and 2000 h. From 6% to 11% of the total daily capture of screwworms could be expected in any 1 hourly period (vertical bars, FIG. 4). A total of 186 female screwworms were captured and 15,371 secondary screwworms were captured (combined sexes).

The relationship between temperature and activity of recaptured female screwworms is shown in FIG. 5 (see methods for treatment of data). The 3rd-order polynomial:

$$\% \text{ capture} = 152 - 20.2T + 0.856T^2 - 0.0114T^3,$$

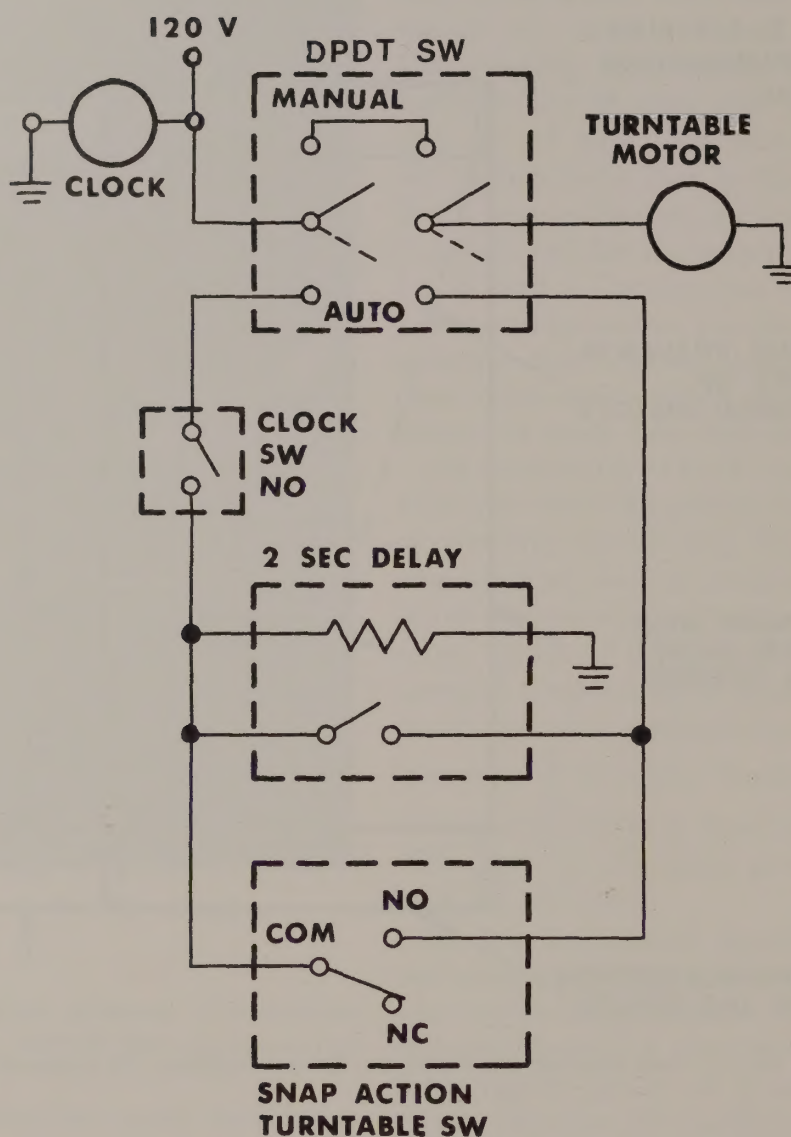


FIG. 3. Schematic drawing of the electrical circuit used to control the time-interval advance mechanism (Abbreviations: DPDT, Double Pole Double Throw; SW, Switch; NO, Normally Open; COM, Common; NC, Normally Closed).

described the data quite closely ($R^2 = 0.76$). Captures increased sharply at temperatures between 20° and 28 °C, followed by a decrease at temperatures above 33 °C. These findings with actual catch data agree quite well with the predictions of Rahn & Barger (1973). Using screwworm case incidence, they suggested that some of the variability (in relationships of case incidence to precipitation) "... was believed to be due to a temperature factor, since insect activity appeared to be diminished by extremely high temperatures." They suggested a critical threshold temperature of ca 35 °C.

The relationship of mean percentage capture of secondary screwworms and temperature (FIG. 6) shows captures at considerably lower temperatures than for screwworms (i.e., at temperatures as low as 12 °C) as well as throughout the temperature

range at which screwworms were captured. Captures increased quadratically with temperature throughout the temperature range experienced; they did not decrease at the highest temperatures like the screwworm captures did. The best-fit polynomial was ($R^2 = 0.90$):

$$\% \text{ capture} = 1.11 - 0.169T + 0.0117T^2.$$

If activity was highly dependent on temperature, we would expect similar activity patterns among days having similar temperature patterns. Therefore, we compared screwworm and secondary screwworm activity on successive days having very similar temperature and RH patterns. This was done first for 2 successive days of relatively high activity (FIG. 7A) and then for 2 successive days of relatively low activity (FIG. 7B).

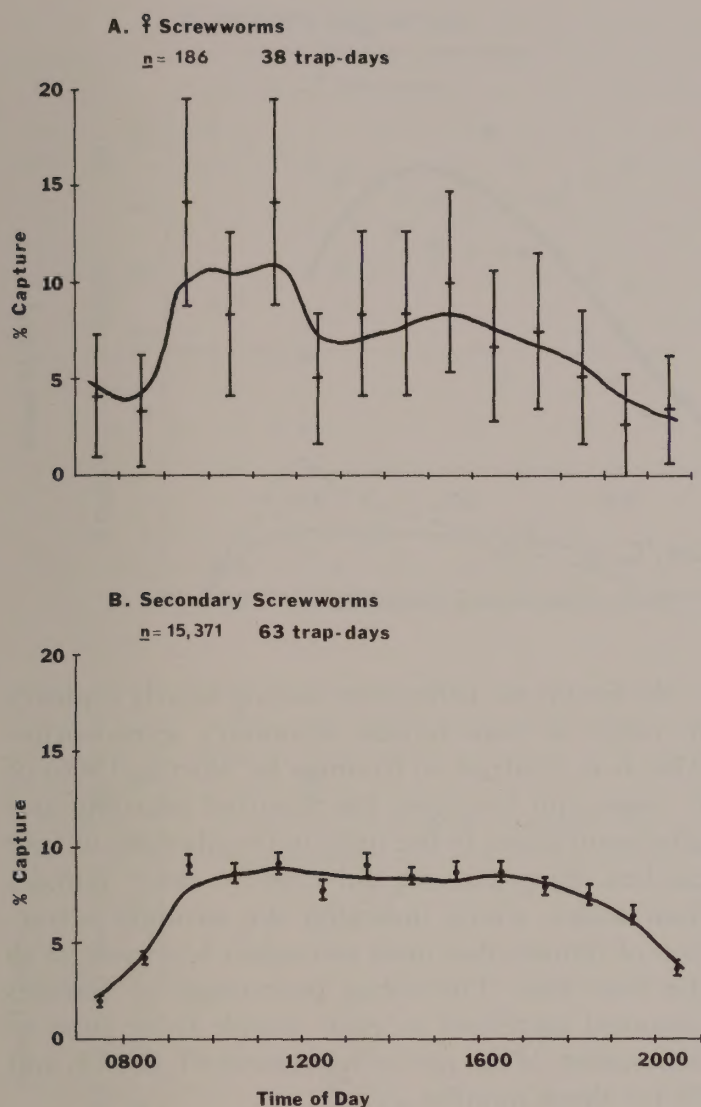


FIG. 4. Mean daily activity as indexed by attraction to decaying liver: A, Mass-reared, released, and recaptured ♀ screwworms; B, Secondary screwworms (combined sexes). Vertical lines denote variability among days in hourly capture data (binomial confidence limits).

When relatively high numbers of flies were captured (in moderate weather) (FIG. 7A), peak captures of screwworms followed fairly similar patterns, peaking at ca 1000 to 1200 h both days. Secondary screwworm captures were much more uniform the 1st day than they were the 2nd day.

With relatively low secondary screwworm captures and no screwworm capture (FIG. 7B—note that these captures were made at slightly lower temperatures and relative humidities than those shown in FIG. 7A), peak secondary screwworm capture occurred during late afternoon the 1st day (3 Oct.) but during midmorning the 2nd day (4 Oct.).

An estimate of variability in captures among all days having 30 °C mean temperature ($\bar{T}$) (not necessarily equal temperature patterns as used above)

is shown in FIG. 8. Data for this $\bar{T}$ only are shown because this provided the largest and thus perhaps the most representative sample (12 days). There was a high amount of variability among days at every hour for screwworms. However, there was no significant difference among hourly captures. But as was true for the overall activity curves (FIG. 4) the variability for screwworms was considerably higher than for secondary screwworms. This was probably related in (an unknown) part to the considerably smaller sample size. For secondary screwworms, there was no significant difference among hourly captures from 0900 to 2000 h.

On days of equal mean temperature, curves of secondary screwworms represented daily captures of 75/day (at $\bar{T} = 23$ °C, 5 days) to 473/day (at $\bar{T} = 31$ °C, 8 days). These captures at mean daily temperatures of 23–30 °C were not highly influenced by time of day, except when lower temperatures probably suppressed activity. Mean capture of secondary screwworms increased linearly with increasing mean daily temperature (correlation coefficient $r = 0.63$) within the range experienced during this experiment (23–31 °C). Although mean temperatures in excess of 31 °C were not experienced, these curves show that mean temperatures in excess of 31 °C would have been required for significant suppression of secondary screwworm activity. Screwworm captures were too low to compare among days of equal $\bar{T}$.

Seasonal trends in activity patterns varied from narrow activity patterns during the cooler, shorter fall days (we caught none of either species in mid-winter) and early spring, to broader patterns during the warmer, longer days of spring, summer, and early fall. Activity of both species decreased during midday and early afternoon on the hottest days. Norris (1966) suggested (in work on other blow flies) that this type of decrease may be associated with solar radiation.

Relative humidity was not closely correlated with captures of female screwworms and secondary screwworms. Second-order regression of percentage capture of female screwworms on RH showed an R^2 of 0.27; for secondary screwworms, R^2 was slightly lower (0.23). A major limitation in determining unbiased relationships of capture and RH was that the maximum daily RH normally occurred in early morning and the minimum occurred about midafternoon (RH is dependent on temperature as well as moisture). Therefore, the lowest RH occurred at periods of highest diurnal activity, and highest RH occurred at periods of lowest activ-

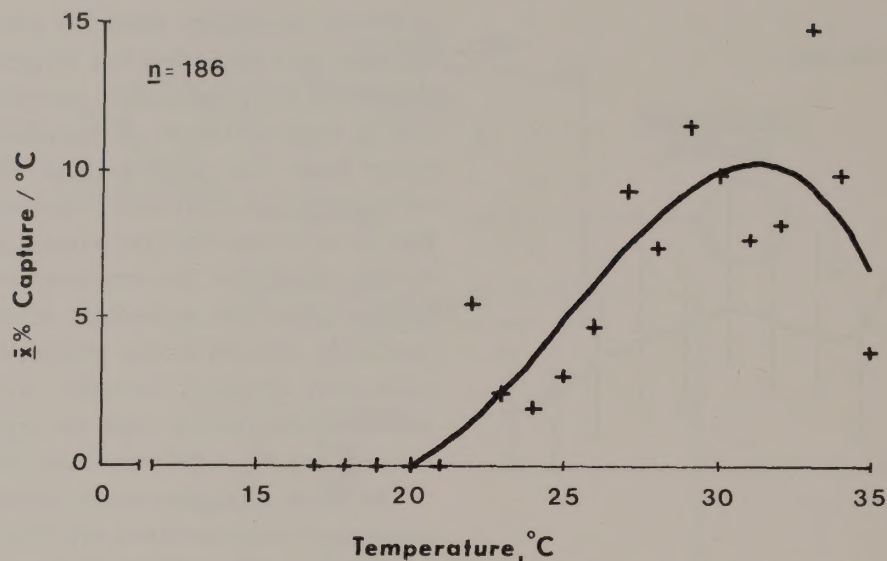


FIG. 5. Influence of temperature on capture of mass-reared, released, and recaptured ♀ screwworms.

ity. This would appear to insure a high correlation (although superinduced), but none was found. Thus, we feel that activity was affected little by humidity. Although we could not investigate the effect of humidity at the extremes, catches were zero or extremely low during rains.

The dissection of female screwworms (we found that 60–80% of the capture was alive) showed that ca 72% of the female screwworms were mated (34 of 47). Thus, a significant number were not mated (13, or 28%), and female screwworms did not come to the liver solely to find an oviposition site. Only 6 screwworm males were collected during this period (all identified as mass-reared). The very low proportion of males captured (2% of total) is in agreement with findings of other investigators of screwworms (e.g., Jones et al. 1976) that very few male screwworms are attracted to decaying liver.

We found no differences among hourly captures in ratios of male:female secondary screwworms. This is in contrast to findings by Norris (1966) of *C. stygia* and *C. augur*. He reported morning and afternoon peaks in the male to female ratio of trap catches. However, we did capture more females than males, which indicated the stronger attraction of female than male secondary screwworms to the liver bait. The mean percentage of females captured increased in each month from June to September. Mean percentages were 67, 69, 72, and 76 for those months.

DISCUSSION

We found the daily activity pattern of both screwworms and secondary screwworms to be quite variable, both with time of day and among

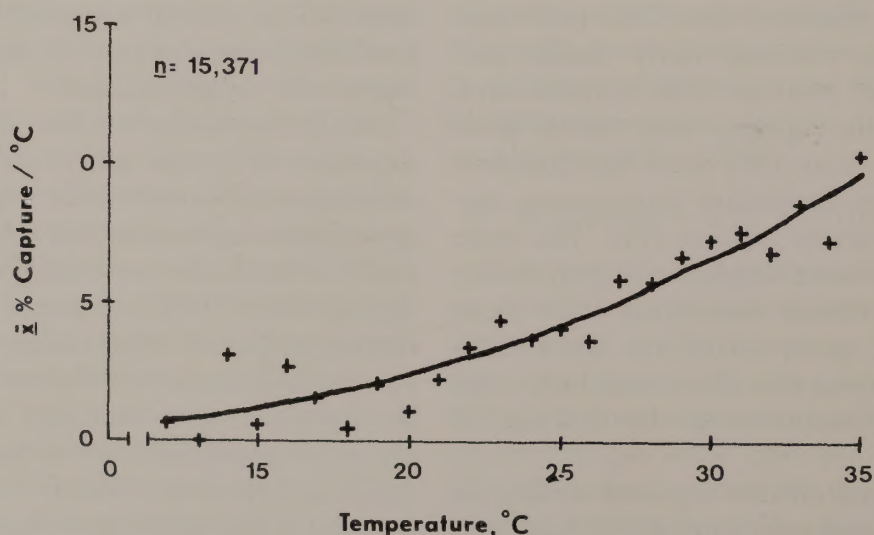


FIG. 6. Influence of temperature on capture of secondary screwworms (combined sexes).

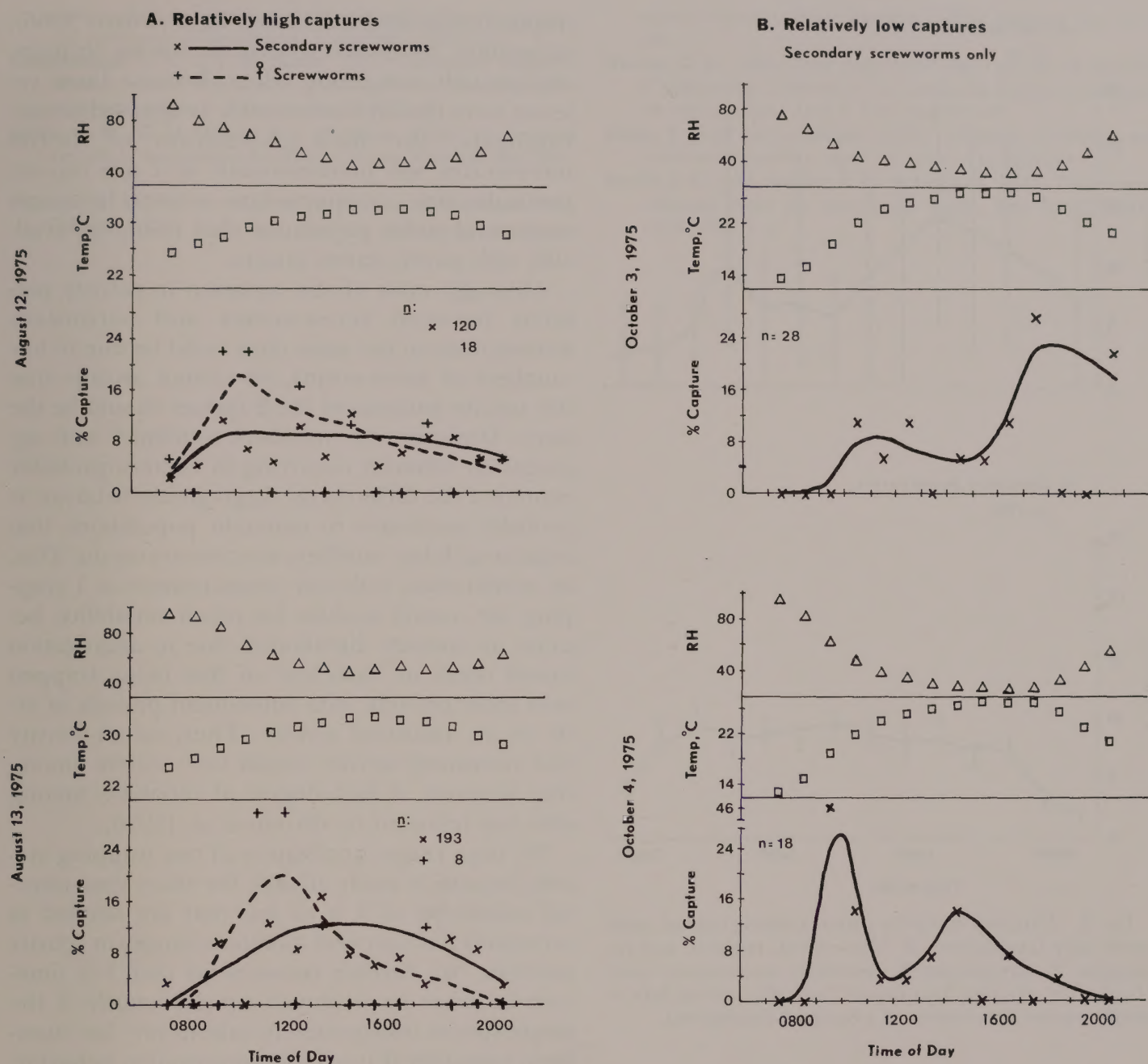


FIG. 7. Captures of screwworms and secondary screwworms on consecutive days of similar temperature and RH patterns: A, Relatively high captures; B, Relatively low captures.

days. Each measurable daily activity pattern was uniquely determined by its own particular set of parameters. Screwworm activity was much more variable than secondary screwworm activity. The number of screwworms captured was a small percentage of the number of secondary screwworms captured. The variability is attributable, in part, to this difference. Captures were significantly correlated with temperature. Lower correlations were found with RH.

Temperature was more influential (assuming sufficient daylight for flight) in affecting activity at temperature extremes than at moderate tempera-

tures—i.e., either low or high temperature would be more likely to suppress activity than moderate temperatures would. The variability in temperatures at which activity begins must be the result of a combination of many factors: among them light (Norris 1966), time of day, and a factor Digby (1958) called adaptation (persisting effects from conditions previously experienced). Adaptation probably exerts its strongest influence in this regard in periods of cool weather. For instance, a gravid female needing an ovipositional site would probably fly sooner (and thus perhaps at either lower or higher temperatures) than a physiologi-

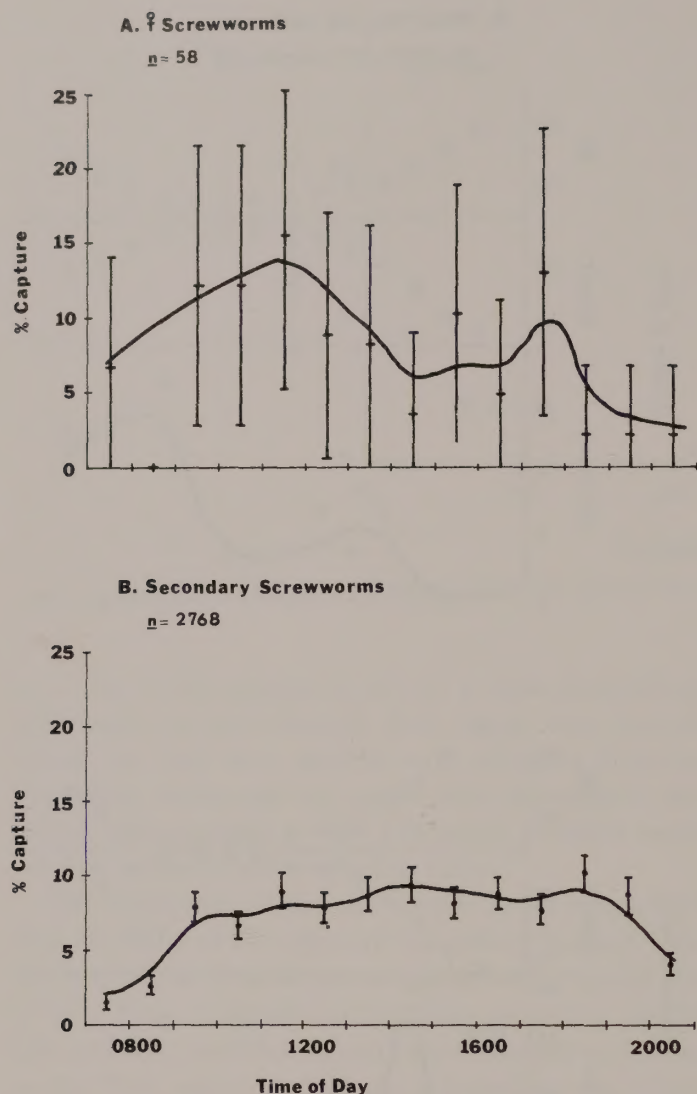


FIG. 8. Variation in activity patterns among days of equal mean daily temperature: A, Mass-reared, released, and recaptured ♀ screwworms; B, Secondary screwworms (combined sexes). Vertical lines denote variability among days in hourly capture data (assuming a binomial distribution).

cally young female with no apparent need to find a host or food.

When the screwworm population is very low (as it frequently is), capture of 1 or 2 flies influences daily activity patterns greatly. The measured pattern is thus shifted significantly when a group of flies or even 1 fly is trapped during a short time interval. The absence of capture does not preclude activity (i.e., flies may be active but not being trapped for a number of reasons, including trap location).

Sample size was dependent on the population available for capture and the efficiency of the sampling device. Additional candidate factors were population age (mainly physiological age of the individual insects as related to susceptibility to being

trapped), day length (season), light (Norris 1966), adaptation, sterile screwworm fly release strategy, and possibly competing odors. Because these releases were usually made weekly as opposed to continuously, the field population of sterile screwworms was predominantly of 1 age on any particular day, as opposed to a more heterogeneous and stable population that would be available with purely native insects.

Although most of the variation in activity patterns between screwworms and secondary screwworms on the same days could be due to low numbers of screwworms, we cannot assume that the activity patterns of the 2 species should be the same. However, low numbers combined with aggregation behavior occurring in Diptera probably extended the differences. Aggregation behavior is probably necessary to maintain populations that exist in such low numbers as screwworms do. This, in combination with our measurement at 1 trapping site, would account for much variability, because an uneven distribution due to aggregation would result in "bunches" of flies being trapped over short periods, with subsequent periods of little or no measured activity. Thus, actual activity and measured activity would vary widely among trap locations. A high degree of variability among sites was reported by Ahrens et al. (1976).

We urge future application of our trapping system, because it easily affords the short time-interval collections of 1 h or less that are needed to accurately discover and monitor changes in activity patterns. We further recommend that 3–5 time-interval traps be deployed simultaneously if the target species is suspected to exist in very low numbers, especially if it exhibits aggregation behavior.

Acknowledgments. We thank H. D. Petersen (USDA, ARS, College Station, TX) for advice and counsel concerning statistical calculations, and technicians of this laboratory, Mr A. J. Siebenaler (for extended analysis and display of data) and Mr F. C. Gonzalez (for constructing the trap and making many of the collections). Others instrumental in data collections were Mr J. J. Garcia of this laboratory, Mr J. J. Garza, presently of USDA, ARS, Falcon Heights, TX, and Mr Fred Perez and Mrs Andrea Fuentes, presently of USDA, APHIS, Mission, TX. We thank Drs A. B. Broce and J. R. Coppedge of this laboratory for critical reviews of the manuscript, and Dr H. D. Petersen and Mr J. S. Smith, Jr (USDA, ARS, Byron, Georgia) for the peer reviews.

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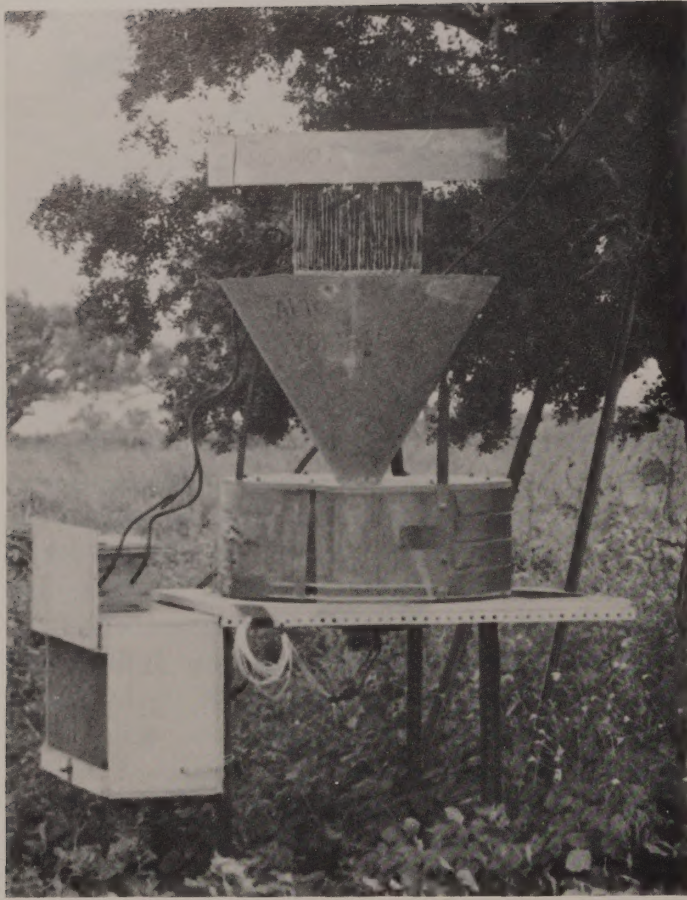


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(DPDT) switch (SW) to auto position. For manual advance of the turntable, DPDT SW was moved to manual position.

Captures of screwworms and secondary screwworms were made during October and November 1974 and February and March 1975 in Kenedy County, Texas and from June to November 1975 in southern Hidalgo County, Texas. The entire screwworm capture and a portion of the secondary screwworm capture were sexed. A portion of the screwworm and secondary screwworm females captured from 10 to 28 Oct. 1974 was checked for mating by microscopic examination of spermatheca squashes. Screwworm flies were identified as native or mass-reared by size and color, a highly subjective procedure that is prone to error. However, too few native flies were captured to be included separately herein.

Collections were usually made daily except on weekends. This provided valid hourly capture data Tuesday through Thursday. Data included in the activity, temperature, and humidity relationships were those obtained for June to November 1975. Data for days of incomplete daily captures (e.g., data not available every hour throughout the day),

trap malfunction, zero daily catch of a particular species, etc., were not used. Of a possible total of 158 trapping days, usable screwworm data were available for 38 days and secondary screwworm data for 63 days. The number of trapping days per month from June to November 1975, respectively, was as follows: for screwworms 7, 9, 12, 5, 2, and 3; and for secondary screwworms 7, 12, 12, 11, 16, and 5. Capture data were coded to $\log(x_i + 1)$ for activity and to $\sqrt{x_i}$ for correlation with temperature and relative humidity to normalize the data. (The choice of $\log$ or $\sqrt{}$ was at our convenience—it made little difference in the curves).

Air temperature (T) ($^{\circ}\text{C}$) and RH were monitored with a Belfort[®] hygrothermograph operated ca 3 m from the interval trap. Mean hourly air temperature and RH were correlated with hourly captures (x_i) of screwworms and secondary screwworms. Because values of temperature and RH occurred at unequal frequencies, a mean capture per occurrence (w_i) was calculated for each 1°C and 1% RH (i.e., $w_i = \sum_{j=1}^{n_i} \{\sqrt{x_i}/n\}$). Finally, the percentage per temperature class was determined ($\%_i = \{w_i/\sum w_i\} \cdot 100$). For RH, the same procedure was used for each 1% RH class.

Best-fit polynomial equations relating activity and temperature or RH were determined by a stepwise regression procedure. The order of equations included herein is that in which the next term (next higher power of x) added did not significantly increase the coefficient of determination (R^2) of the equation (t -test, $P < 0.05$).

An additional temperature comparison was made by finding percentage/class as described above, but for each hourly capture period separately (i.e., we constructed activity vs temperature correlations for captures from 0800 to 0900 h and then separately for 0900–1000 h, etc). This was done to try to adjust for the fact that all temperatures did not occur an equal number of times at each hour of the day. This covariance procedure involved a considerable amount of work but did not increase the correlation of capture and temperature or capture and RH. We mention it here so that others may avoid this fruitless effort.

Lastly, we determined estimates of variability in captures among days of equal (to the nearest $^{\circ}\text{C}$) mean temperature. Mean daily temperatures were calculated from mean hourly temperatures (those used in the correlations with hourly captures) from 0700 to 2100 h daily. Standard deviations of captures were calculated from hourly catches of both

**SCALE: 1/2 SIZE
ALL DIMENSIONS
IN CM**

**FUNNEL PREVENTS
ESCAPE OF
CAPTURED INSECTS**

**CYLINDER AND
FUNNEL WIRE
MESH SCREEN**

**REMOVABLE BOTTOM
(FRUIT JAR COVER)**

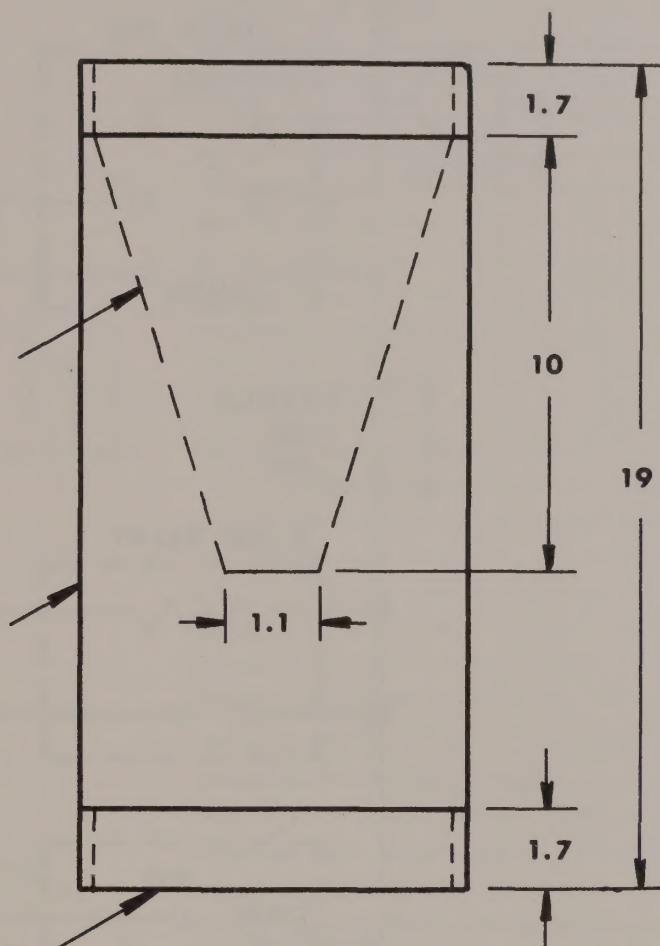


FIG. 2. Line drawing of a modified collection container (15 required).

species on these days. Hourly captures were coded to $\log(x_i + 1)$ for these determinations.

RESULTS

Mean activity and estimated variability of activity of mass-reared female screwworm and secondary screwworm (both sexes combined) flies are shown in FIG. 4. The smooth curves are hand drawn. Vertical bars denote ca 95% confidence intervals (a binomial distribution was assumed). Both species were caught throughout the day, but secondary screwworms were caught in much higher numbers. Screwworm captures were highest in the morning, with a possible secondary peak in the afternoon. Screwworm captures from 0900 to 1000 h and 1100 to 1200 h were significantly higher (t -test, $P < 0.05$) than at 1200 to 1300 h, but not higher than any others except those from 0700 to 0900 h or after 1800 h. Norris (1966) reported pronounced morning and afternoon peaks in trap catches of *Calliphora stygia* (F.) and *C. augur* (F.) in hot weath-

er, but unimodal curves of trap catches in colder months. We suspect similar trends, but captures were too low in winter to determine if curves were truly unimodal. It was unfortunate that native female screwworms were captured in numbers too limited for comparison of their activity with that of recaptured sterilized mass-reared females. There was little difference in secondary screwworm activity from 0900 to 1900 h. Captures before 0900 h and after 2000 h were significantly lower than between 0900 and 2000 h. From 6% to 11% of the total daily capture of screwworms could be expected in any 1 hourly period (vertical bars, FIG. 4). A total of 186 female screwworms were captured and 15,371 secondary screwworms were captured (combined sexes).

The relationship between temperature and activity of recaptured female screwworms is shown in FIG. 5 (see methods for treatment of data). The 3rd-order polynomial:

$$\% \text{ capture} = 152 - 20.2T + 0.856T^2 - 0.0114T^3,$$

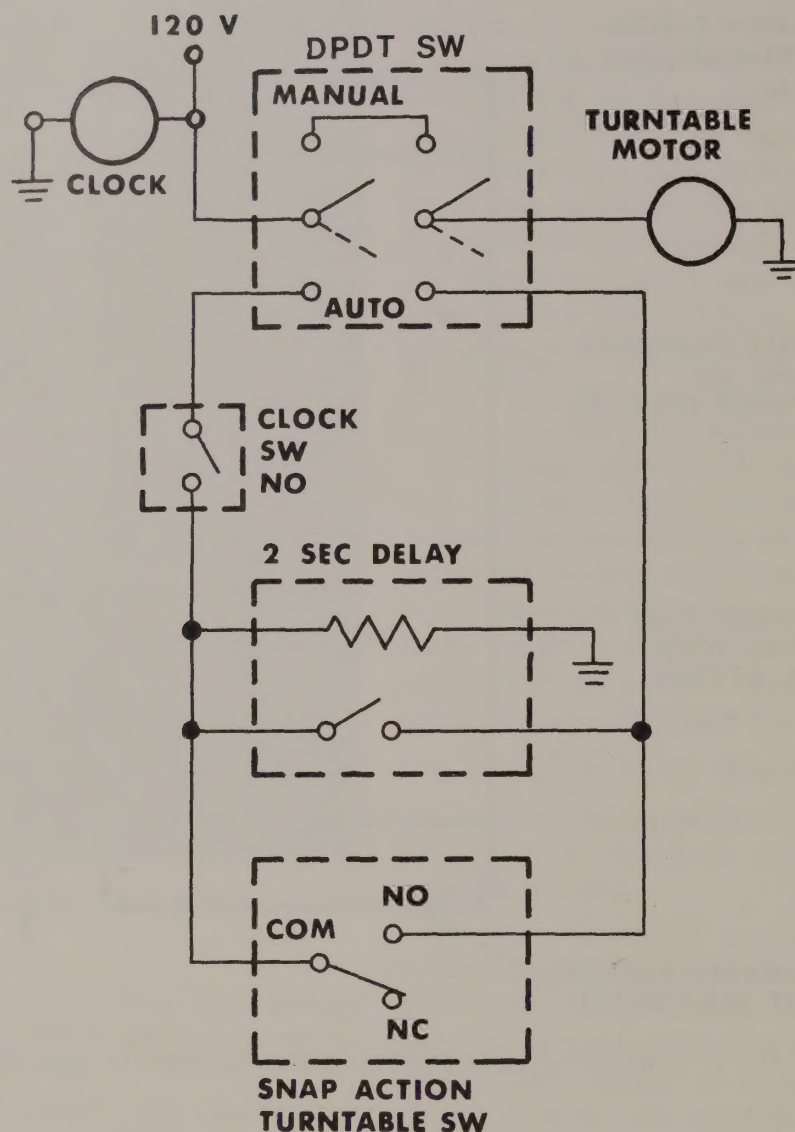


FIG. 3. Schematic drawing of the electrical circuit used to control the time-interval advance mechanism (Abbreviations: DPDT, Double Pole Double Throw; SW, Switch; NO, Normally Open; COM, Common; NC, Normally Closed).

described the data quite closely ($R^2 = 0.76$). Captures increased sharply at temperatures between 20° and 28 °C, followed by a decrease at temperatures above 33 °C. These findings with actual catch data agree quite well with the predictions of Rahn & Barger (1973). Using screwworm case incidence, they suggested that some of the variability (in relationships of case incidence to precipitation) "... was believed to be due to a temperature factor, since insect activity appeared to be diminished by extremely high temperatures." They suggested a critical threshold temperature of ca 35 °C.

The relationship of mean percentage capture of secondary screwworms and temperature (FIG. 6) shows captures at considerably lower temperatures than for screwworms (i.e., at temperatures as low as 12 °C) as well as throughout the temperature

range at which screwworms were captured. Captures increased quadratically with temperature throughout the temperature range experienced; they did not decrease at the highest temperatures like the screwworm captures did. The best-fit polynomial was ($R^2 = 0.90$):

$$\% \text{ capture} = 1.11 - 0.169T + 0.0117T^2.$$

If activity was highly dependent on temperature, we would expect similar activity patterns among days having similar temperature patterns. Therefore, we compared screwworm and secondary screwworm activity on successive days having very similar temperature and RH patterns. This was done first for 2 successive days of relatively high activity (FIG. 7A) and then for 2 successive days of relatively low activity (FIG. 7B).

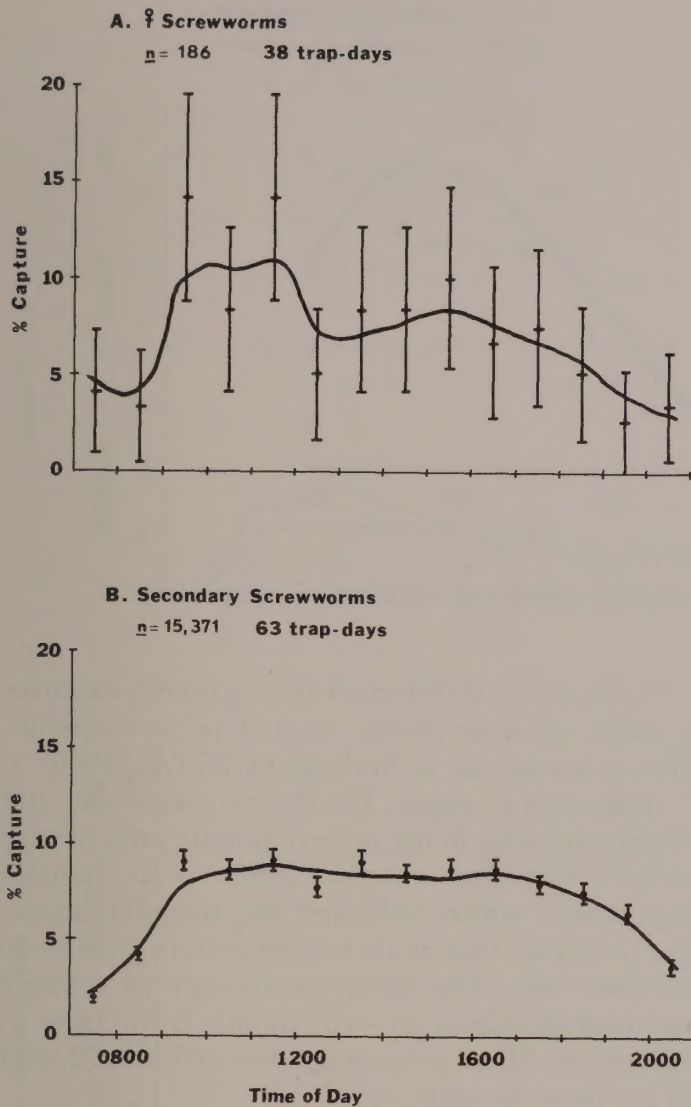


FIG. 4. Mean daily activity as indexed by attraction to decaying liver: A, Mass-reared, released, and recaptured ♀ screwworms; B, Secondary screwworms (combined sexes). Vertical lines denote variability among days in hourly capture data (binomial confidence limits).

When relatively high numbers of flies were captured (in moderate weather) (FIG. 7A), peak captures of screwworms followed fairly similar patterns, peaking at ca 1000 to 1200 h both days. Secondary screwworm captures were much more uniform the 1st day than they were the 2nd day.

With relatively low secondary screwworm captures and no screwworm capture (FIG. 7B—note that these captures were made at slightly lower temperatures and relative humidities than those shown in FIG. 7A), peak secondary screwworm capture occurred during late afternoon the 1st day (3 Oct.) but during midmorning the 2nd day (4 Oct.).

An estimate of variability in captures among all days having 30 °C mean temperature ($\bar{T}$) (not necessarily equal temperature patterns as used above)

is shown in FIG. 8. Data for this $\bar{T}$ only are shown because this provided the largest and thus perhaps the most representative sample (12 days). There was a high amount of variability among days at every hour for screwworms. However, there was no significant difference among hourly captures. But as was true for the overall activity curves (FIG. 4) the variability for screwworms was considerably higher than for secondary screwworms. This was probably related in (an unknown) part to the considerably smaller sample size. For secondary screwworms, there was no significant difference among hourly captures from 0900 to 2000 h.

On days of equal mean temperature, curves of secondary screwworms represented daily captures of 75/day (at $\bar{T} = 23$ °C, 5 days) to 473/day (at $\bar{T} = 31$ °C, 8 days). These captures at mean daily temperatures of 23–30 °C were not highly influenced by time of day, except when lower temperatures probably suppressed activity. Mean capture of secondary screwworms increased linearly with increasing mean daily temperature (correlation coefficient $r = 0.63$) within the range experienced during this experiment (23–31 °C). Although mean temperatures in excess of 31 °C were not experienced, these curves show that mean temperatures in excess of 31 °C would have been required for significant suppression of secondary screwworm activity. Screwworm captures were too low to compare among days of equal $\bar{T}$.

Seasonal trends in activity patterns varied from narrow activity patterns during the cooler, shorter fall days (we caught none of either species in mid-winter) and early spring, to broader patterns during the warmer, longer days of spring, summer, and early fall. Activity of both species decreased during midday and early afternoon on the hottest days. Norris (1966) suggested (in work on other blow flies) that this type of decrease may be associated with solar radiation.

Relative humidity was not closely correlated with captures of female screwworms and secondary screwworms. Second-order regression of percentage capture of female screwworms on RH showed an R^2 of 0.27; for secondary screwworms, R^2 was slightly lower (0.23). A major limitation in determining unbiased relationships of capture and RH was that the maximum daily RH normally occurred in early morning and the minimum occurred about midafternoon (RH is dependent on temperature as well as moisture). Therefore, the lowest RH occurred at periods of highest diurnal activity, and highest RH occurred at periods of lowest activ-

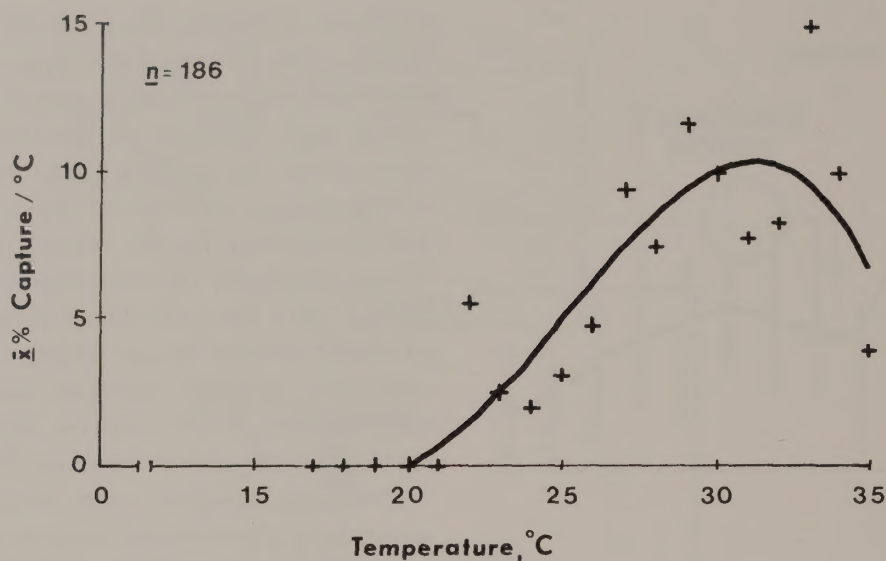


FIG. 5. Influence of temperature on capture of mass-reared, released, and recaptured ♀ screwworms.

ity. This would appear to insure a high correlation (although superinduced), but none was found. Thus, we feel that activity was affected little by humidity. Although we could not investigate the effect of humidity at the extremes, catches were zero or extremely low during rains.

The dissection of female screwworms (we found that 60–80% of the capture was alive) showed that ca 72% of the female screwworms were mated (34 of 47). Thus, a significant number were not mated (13, or 28%), and female screwworms did not come to the liver solely to find an oviposition site. Only 6 screwworm males were collected during this period (all identified as mass-reared). The very low proportion of males captured (2% of total) is in agreement with findings of other investigators of screwworms (e.g., Jones et al. 1976) that very few male screwworms are attracted to decaying liver.

We found no differences among hourly captures in ratios of male:female secondary screwworms. This is in contrast to findings by Norris (1966) of *C. stygia* and *C. augur*. He reported morning and afternoon peaks in the male to female ratio of trap catches. However, we did capture more females than males, which indicated the stronger attraction of female than male secondary screwworms to the liver bait. The mean percentage of females captured increased in each month from June to September. Mean percentages were 67, 69, 72, and 76 for those months.

DISCUSSION

We found the daily activity pattern of both screwworms and secondary screwworms to be quite variable, both with time of day and among

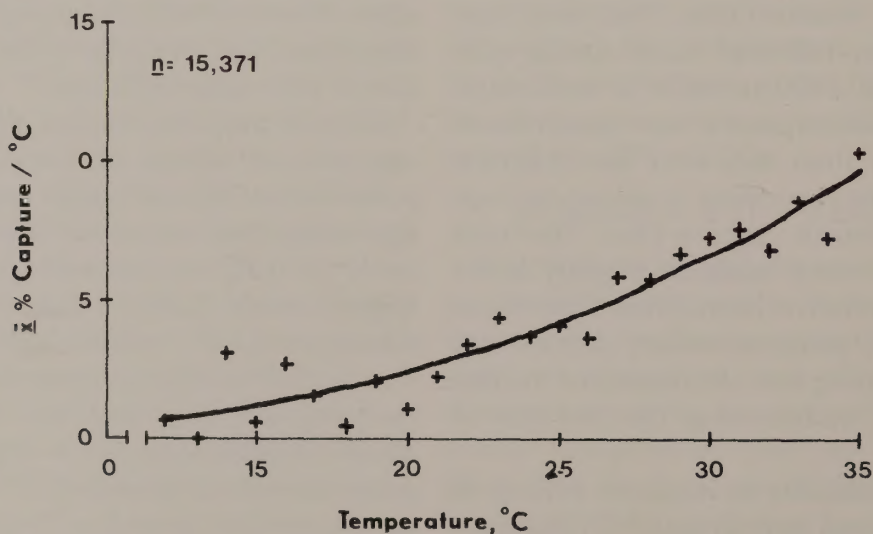


FIG. 6. Influence of temperature on capture of secondary screwworms (combined sexes).

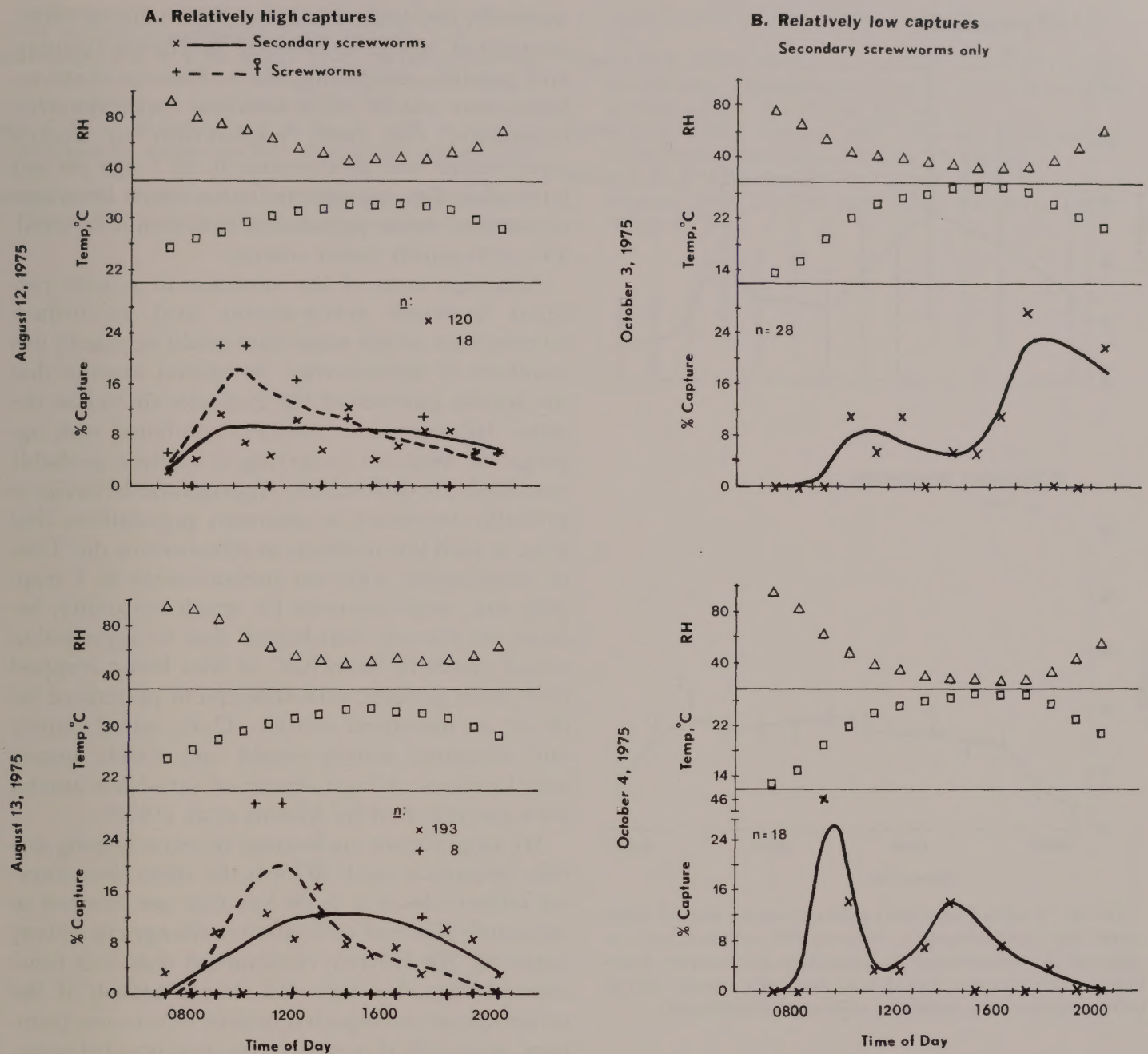


FIG. 7. Captures of screwworms and secondary screwworms on consecutive days of similar temperature and RH patterns: A, Relatively high captures; B, Relatively low captures.

days. Each measurable daily activity pattern was uniquely determined by its own particular set of parameters. Screwworm activity was much more variable than secondary screwworm activity. The number of screwworms captured was a small percentage of the number of secondary screwworms captured. The variability is attributable, in part, to this difference. Captures were significantly correlated with temperature. Lower correlations were found with RH.

Temperature was more influential (assuming sufficient daylight for flight) in affecting activity at temperature extremes than at moderate tempera-

tures—i.e., either low or high temperature would be more likely to suppress activity than moderate temperatures would. The variability in temperatures at which activity begins must be the result of a combination of many factors: among them light (Norris 1966), time of day, and a factor Digby (1958) called adaptation (persisting effects from conditions previously experienced). Adaptation probably exerts its strongest influence in this regard in periods of cool weather. For instance, a gravid female needing an ovipositional site would probably fly sooner (and thus perhaps at either lower or higher temperatures) than a physiologi-

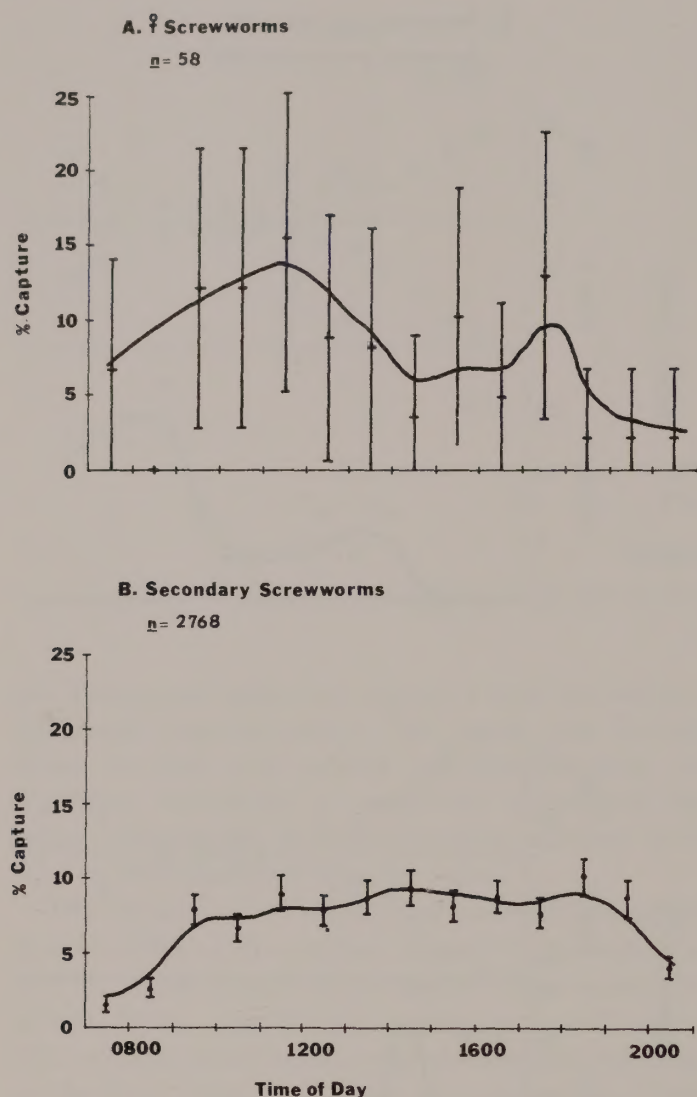


FIG. 8. Variation in activity patterns among days of equal mean daily temperature: A, Mass-reared, released, and recaptured ♀ screwworms; B, Secondary screwworms (combined sexes). Vertical lines denote variability among days in hourly capture data (assuming a binomial distribution).

cally young female with no apparent need to find a host or food.

When the screwworm population is very low (as it frequently is), capture of 1 or 2 flies influences daily activity patterns greatly. The measured pattern is thus shifted significantly when a group of flies or even 1 fly is trapped during a short time interval. The absence of capture does not preclude activity (i.e., flies may be active but not being trapped for a number of reasons, including trap location).

Sample size was dependent on the population available for capture and the efficiency of the sampling device. Additional candidate factors were population age (mainly physiological age of the individual insects as related to susceptibility to being

trapped), day length (season), light (Norris 1966), adaptation, sterile screwworm fly release strategy, and possibly competing odors. Because these releases were usually made weekly as opposed to continuously, the field population of sterile screwworms was predominantly of 1 age on any particular day, as opposed to a more heterogeneous and stable population that would be available with purely native insects.

Although most of the variation in activity patterns between screwworms and secondary screwworms on the same days could be due to low numbers of screwworms, we cannot assume that the activity patterns of the 2 species should be the same. However, low numbers combined with aggregation behavior occurring in Diptera probably extended the differences. Aggregation behavior is probably necessary to maintain populations that exist in such low numbers as screwworms do. This, in combination with our measurement at 1 trapping site, would account for much variability, because an uneven distribution due to aggregation would result in "bunches" of flies being trapped over short periods, with subsequent periods of little or no measured activity. Thus, actual activity and measured activity would vary widely among trap locations. A high degree of variability among sites was reported by Ahrens et al. (1976).

We urge future application of our trapping system, because it easily affords the short time-interval collections of 1 h or less that are needed to accurately discover and monitor changes in activity patterns. We further recommend that 3–5 time-interval traps be deployed simultaneously if the target species is suspected to exist in very low numbers, especially if it exhibits aggregation behavior.

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Screwworm Flies:¹ Seasonal Occurrence in Central Tamaulipas, Mexico, 1973-74²

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ABSTRACT

A series of 13 traps was operated in central Tamaulipas, Mexico, to monitor the population of *Cochliomyia hominivorax* (Coquerel) through a complete season. Adult

screwworm flies were captured in all but 3 weeks. Peak captures occurred during the 10-week period, Aug. 17 to Oct. 24; lowest captures occurred in April, May, and June.

In 1972, the United States signed an agreement with Mexico to extend the screwworm barrier to the Isthmus of Tehuantepec located in the narrowest part of Mexico (in the State of Chiapas). In preparation for this program, we studied the seasonal trends of the screwworm population in central Tamaulipas, Mexico.

METHODS AND MATERIALS.—On April 19, 1973, 13 conical fly traps were placed on an east-west line along a highway from a point 12 km W of Ciudad Victoria eastward through Sota la Marina to a point near LaPesca, a distance of 175 km. The traps were placed from 6–12 km apart, and the altitude at the trap sites was near sea level on the east, 600 m in the Tamaulipas Mountains (midway of the trap line), 300 m between the Tamaulipas Mountains and Ciudad Victoria, and 600 m in the edge of the Sierra Madre Oriental west of Ciudad Victoria. The traps were in operation until April 15, 1974.

Conical fly traps (Bishopp 1916) of a collapsible type being used in screwworm research and control programs were baited with 2 lb fresh bovine liver

in sufficient water to leave $\frac{1}{4}$ – $\frac{1}{2}$ of it exposed. At the end of the 1st week, $\frac{1}{2}$ the liver and all the water were discarded and replaced. Each week thereafter, the oldest piece of liver was replaced along with the water. Flooding in a canyon sometimes prevented the servicing of the most western trap. Also, an impassable road following rains occasionally prevented servicing of 3 traps in the coastal area to the east. Flies accumulated in a trap that was not serviced the previous week were discarded because attractiveness of bait more than one week old is not comparable to that of bait renewed each week. In addition, when a trap had been disturbed (down, damaged, missing, bait pan upset, etc.), the catch, if any, was discarded.

RESULTS AND DISCUSSION.—The weekly averages are shown in Fig. 1 and are based on as few as 8 (2 times) to all 13 traps (8 times). The average number of traps contributing per week for the 51 weeks was 11.3. Screwworm flies were captured in all but 3 of the 51 weeks—the 1st week of the study and a 2-week period beginning June 26. The average number of flies captured per trap per week varied from 0–13.3. This average was 4 or below for 31 weeks and exceeded 10 during only 2 weeks. The greatest catch in 1973 occurred during a 10-week period from Aug. 17 to Oct. 24. In 1974, the catch exceeded 4/trap/week from Jan. 23 through the remainder of the study. The largest single catch in one trap was 5 ♂ and 98 ♀ taken during the 1st week in September. This trap, which consistently captured more

¹ Diptera: Calliphoridae.

² Received for publication July 22, 1976.

³ Retired December 1974. Present address: Route 6, Box 237-D, Fort Payne, AL 35967.

⁴ Published in cooperation with the Comision Mexico-Americana Para La Erradicacion Del Gusano Barrenador Del Ganado. Dr. Marco A. Villasenor is Director.

⁵ The assistance of technicians Erasmo Lopez, who helped with trap servicing, and Brenda Anthony, who sorted flies, is gratefully acknowledged.

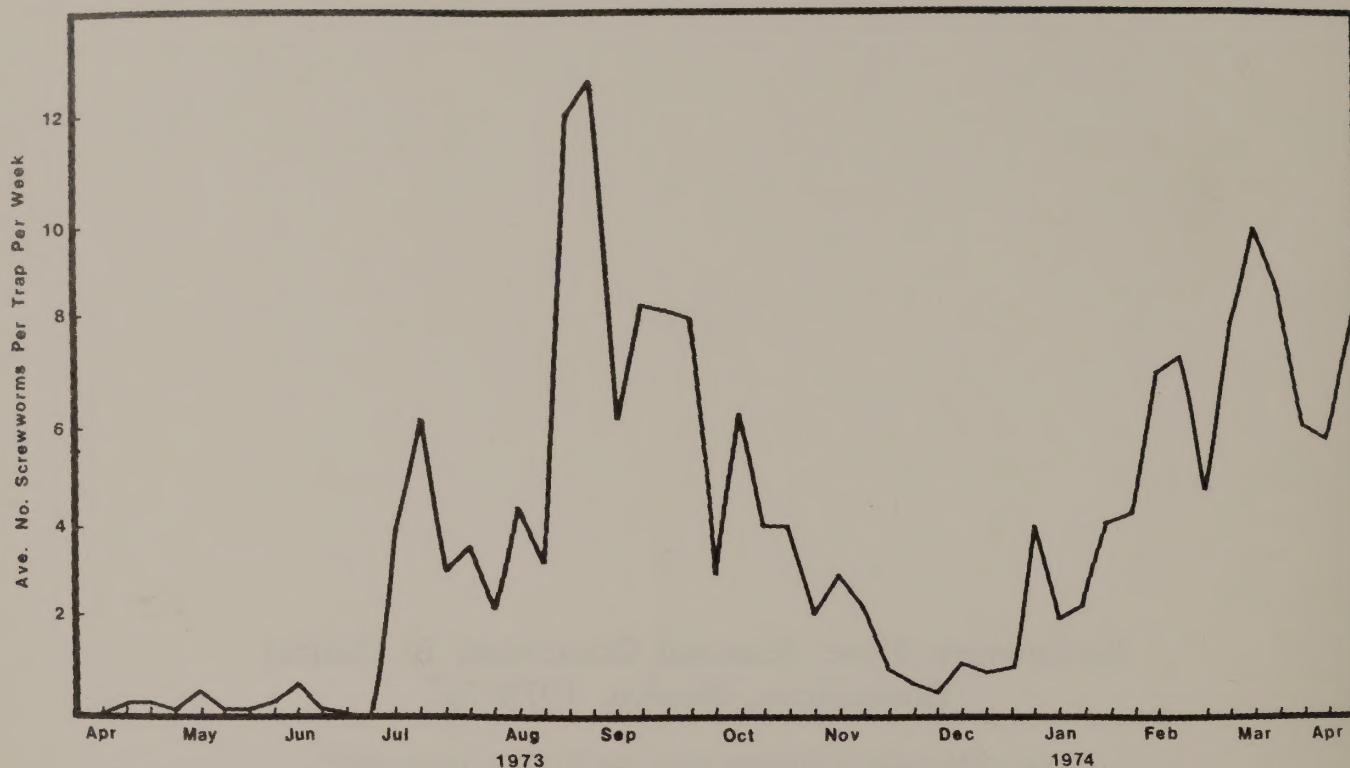


FIG. 1.—Flies captured in liver-baited traps operated for one year in central Tamaulipas, Mexico.

screwworms and other Diptera than any other trap, was located 6 km W of Ciudad Victoria in a canyon that may have served as a flyway for dispersing flies. However, the population of small domestic animals (poultry, swine, and goats) in this area also seemed to be greater than at other trap locations. The high screwworm population in this area is of particular interest since livestock inspectors have reported that the 1st infestation of screwworms in the spring in the Gulf coastal plains of northeastern Mexico are found

near the mouth of canyons in the Sierra Madre Oriental Mountain range.

Of the 2438 screwworm flies captured, 6.6% were males, a sex ratio of 1:15.2. The largest weekly catch of males in one trap was 7. The average number of males per trap per week never exceeded 0.9.

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A Chemical Attractant for Screwworm Flies^{1,2}

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ABSTRACT

A chemical bait that was as attractive as liver to adult *Cochliomyia hominivorax* (Coquerel) contained 10 chemicals found to be degradation products of animal protein. Best results were obtained by separating the chemicals into 3 vials based on compatibility. In a

20-wk comparison of the chemical bait with liver, the bait attracted 5.7 times more ♂ screwworms, and the liver attracted 1.7 times more females and 7.7 times more trash flies.

Bishopp (1916) was the first to recommend the use of traps to reduce the populations of flies causing myiasis in livestock in the Southwest. Subsequently, he and other researchers (Bishopp et al. 1923, Parman et al. 1928, Laake and Cushing 1930, Bruce and Knipling 1936) used decomposing meat or meat by-products in water as baits. The most effective were found to be gut slime, rabbit carcasses, goat and sheep meat, and beef and pork liver. It was not until 1932 that Cushing and Patton (1933) discovered that the primary cause of myiasis in livestock was screwworms, *Cochliomyia hominivorax* (Coquerel), and that *C. macellaria* (F.), *Lucilia sp.*, and *Phormia sp.* were only secondary invaders. This discovery, however, did not influence subsequent researchers to change trap design or baits. During the past 40 yr, virtually all workers trapping screwworm flies have used the Bishopp trap with only slight modifications and beef liver as bait.

In recent years, extensive efforts have been made to find an attractant to replace liver, especially one that attracts males. Thus DeVaney et al. (1973) studied the attractiveness to screwworm flies of bacteria-contaminated bovine blood, bacteria-inoculated sterile blood, defibrinated blood, and blood plasma in laboratory olfactometer tests. They concluded that the attractiveness of the materials resulted from the bacteria or from the compounds produced by them. Later, Grabbe and Turner (1973) isolated and identified phenol, *p*-cresol, and indole as the attractive components of the incubated blood prepared by DeVaney and associates and tentatively identified others. Dilute aqueous mixtures of these compounds were attractive to screwworm flies, but pure individual compounds were not very attractive. Grabbe assembled and tested mixtures of 35 compounds that he considered likely degradation products of animal proteins and fatty acids and demonstrated in the field that the mixtures were attractive to screwworm flies (R. R. Grabbe, unpubl., Screwworm Research Laboratory, ARS, USDA, Mission, TX).

We report here our efforts to develop the chemical mixtures found attractive by Grabbe into a useful field formulation.

TESTS AND RESULTS.—Thirty of the 35 compounds selected by Grabbe were chosen for the study (acetic acid, glacial, benzoic acid, butyric acid, lactic acid, hexanoic acid, octanoic acid, valeric acid, propanoic acid, acetaldehyde, formalin, indole, skatole, phenol, *p*-cresol, pyridine, *n*-butyl alcohol, iso-butyl alcohol, sec-butyl alcohol, acetone, methanol, ethanol, 2-butanone, methyl sulfide, ethanethiol, 1,5-diaminopentane, ethanolamine, ethylamine, methylamine, isopropylamine, trimethylamine). Screening tests were conducted outdoors in natural screwworm fly habitats with the Bishopp-type fly trap presently being used in research and eradication programs. Twenty-eight of these traps were hung in a north-south line 500 m long along the edge of a dense growth of small trees and shrubs that bordered a drainage ditch near the laboratory and the screwworm rearing plant. Sterile flies that escaped from the packaging and loading operations and those released daily in quality control agility tests for the eradication program assured a rather high and stable, though sterile, fly population.

Preliminary tests were made to find a satisfactory method of exposing the chemical baits. First, we attempted to minimize chemical incompatibility. Thus 10 μ l of each of the 6 amine compounds (last 6 chemicals listed) were combined in 10 ml of glycerol, and 10 μ l of each of the remaining 24 chemicals (except 10 mg of the crystalline compounds—benzoic acid, indole and skatole) were combined in 10 ml of glycerol. The glycerol solutions were in separate 10-cm-diam open dishes and placed in bait pans hung beneath the traps. Small but consistent catches of flies prompted us to increase the amount of each chemical per trap to 500 μ l or 500 mg. Also the 24 non-amines were divided into 2 groups, the acids and aldehydes (the 1st 10 chemicals listed) and the alcohols and ketones (the next 14 chemicals listed).

To improve volatilization, ethanol was substituted for glycerol as a solvent. The ethanol solutions were placed in bottles containing wicks. Several trials with various kinds of wicks in combination with different quantities of ethanol indicated that bottles containing 40–50 ml of ethanol and a No. 2 dental roll extending 1.3 cm above the mouth of the bottle provided a steady, prolonged release of the volatile material.

¹ Diptera: Calliphoridae.

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⁷ We thank the following technicians at the Screwworm Research Laboratory for their help in conducting this study: Erasmo Lopez and Francisco C. Gonzalez for trapping, and Brenda K. Anthony for sorting and identifying the screwworm flies.

O. H. Graham

When the 3 groups of chemicals (amines, acids and aldehydes, and alcohols and ketones) were tested separately, the 1st and 2nd groups were very weakly attractive and the 3rd group was moderately attractive to screwworm flies. When combined, groups 2 and 3 complemented each other, but the 3rd group (amines) used in combination with either or both of the other 2 groups markedly decreased the attractiveness of the bait. No further studies were conducted with the amines.

Subsequently, we found that more screwworm flies were trapped when the chemicals were separated from each other by placing a few milliliters without ethanol and wick in a vial and grouping the vials in a bait pan. This method of exposure also simplified the evaluation of single compounds.

Finally, in an effort to categorize the remaining 25 chemicals as attractive, neutral or repellent, we omitted one or a small group of chemicals in tests. A resulting decrease of at least 10% in the number of screwworm flies trapped indicated that the omitted chemical was beneficial; little or no difference in catch suggested that the chemical was neither helpful nor harmful; a larger catch indicated that the omitted chemical was repellent. Whenever a repellent chemical was identified, it was eliminated from further testing, and the remaining mixture was designated the new standard for further testing. Tests were repeated when results were questionable. Although liver was almost always included in a test, the results of each treatment also were compared with the newest chemical standard.

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DISCUSSION.—This study has resulted in the development of a screwworm fly attractant superior to liver in trapping males but less effective in attracting females. However, the 5.7-fold increase in the number of males captured has caused us to use it as the standard bait for trapping screwworms.

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	Chemical bait			Liver						
	Screwworm		Other Diptera	Screwworm		Other Diptera				
	♂	♀		♂	♀					
1	31	511	2,902	20	375	14,054	60.8	57.7	1:16.5	1: 18.8
2	17	102	2,951	25	315	16,253	40.5	24.5	1: 6.0	1: 12.6
3	3	14	391	3	18	3,145	50.0	43.8	1: 4.7	1: 6.0
4	24	34	1,118	1	12	1,338	96.0	73.9	1: 1.4	1: 12.0
5	5	13	816	1	4	3,193	83.3	76.5	1: 2.6	1: 4.0
6	15	7	226	0	6	3,197	100.0	53.8	1: 0.5	1: 6.0
7	10	19	383	0	1	2,558	100.0	95.0	1: 1.9	1: 1.0
8	12	13	1,313	0	10	12,056	100.0	56.5	1: 1.1	1: 10.0
9	0	0	925	0	0	5,456				
10	0	0	1,258	0	0	5,513				
11	3	21	2,175	1	26	22,493	75.0	44.7	1: 7.0	1: 26.0
12	1	0	117	1	0	1,189	50.0		1: 0	1: 0
13	10	115	839	1	360	22,709	90.9	24.2	1:11.5	1:360.0
14	137	911	7,596	32	1162	43,971	81.1	43.9	1: 6.6	1: 36.3
15	59	517	9,998	17	2041	86,372	77.6	20.2	1: 8.8	1:120.0
16	25	353	7,557	2	698	75,859	92.6	33.6	1:14.1	1:349.0
17	163	487	11,589	4	324	94,945	97.6	60.0	1: 3.0	1: 81.0
18	22	236	18,339	0	297	98,365	100.0	44.3	1:10.7	1:297.0
19	122	339	13,636	8	457	75,434	93.8	42.6	1: 2.8	1: 57.1
20	42	308	13,717	7	761	56,473				
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A Chemical Attractant for Screwworm Flies^{1,2}

CALVIN M. JONES,³ DELBERT D. OEHLER,⁴ J. WENDELL SNOW,⁵ and ROLLAND R. GRABBE^{6,7}

ABSTRACT

A chemical bait that was as attractive as liver to adult *Cochliomyia hominivorax* (Coquerel) contained 10 chemicals found to be degradation products of animal protein. Best results were obtained by separating the chemicals into 3 vials based on compatibility. In a

20-wk comparison of the chemical bait with liver, the bait attracted 5.7 times more ♂ screwworms, and the liver attracted 1.7 times more females and 7.7 times more trash flies.

Bishopp (1916) was the first to recommend the use of traps to reduce the populations of flies causing myiasis in livestock in the Southwest. Subsequently, he and other researchers (Bishopp et al. 1923, Parman et al. 1928, Laake and Cushing 1930, Bruce and Knipling 1936) used decomposing meat or meat by-products in water as baits. The most effective were found to be gut slime, rabbit carcasses, goat and sheep meat, and beef and pork liver. It was not until 1932 that Cushing and Patton (1933) discovered that the primary cause of myiasis in livestock was screwworms, *Cochliomyia hominivorax* (Coquerel), and that *C. macellaria* (F.), *Lucilia* sp., and *Phormia* sp. were only secondary invaders. This discovery, however, did not influence subsequent researchers to change trap design or baits. During the past 40 yr, virtually all workers trapping screwworm flies have used the Bishopp trap with only slight modifications and beef liver as bait.

In recent years, extensive efforts have been made to find an attractant to replace liver, especially one that attracts males. Thus DeVaney et al. (1973) studied the attractiveness to screwworm flies of bacteria-contaminated bovine blood, bacteria-inoculated sterile blood, defibrinated blood, and blood plasma in laboratory olfactometer tests. They concluded that the attractiveness of the materials resulted from the bacteria or from the compounds produced by them. Later, Grabbe and Turner (1973) isolated and identified phenol, *p*-cresol, and indole as the attractive components of the incubated blood prepared by DeVaney and associates and tentatively identified others. Dilute aqueous mixtures of these compounds were attractive to screwworm flies, but pure individual compounds were not very attractive. Grabbe assembled and tested mixtures of 35 compounds that he considered likely degradation products of animal proteins and fatty acids and demonstrated in the field that the mixtures were attractive to screwworm flies (R. R. Grabbe, unpubl., Screwworm Research Laboratory, ARS, USDA, Mission, TX).

We report here our efforts to develop the chemical mixtures found attractive by Grabbe into a useful field formulation.

TESTS AND RESULTS.—Thirty of the 35 compounds selected by Grabbe were chosen for the study (acetic acid, glacial, benzoic acid, butyric acid, lactic acid, hexanoic acid, octanoic acid, valeric acid, propanoic acid, acetaldehyde, formalin, indole, skatole, phenol, *p*-cresol, pyridine, *n*-butyl alcohol, iso-butyl alcohol, sec-butyl alcohol, acetone, methanol, ethanol, 2-butanone, methyl sulfide, ethanethiol, 1,5-diaminopentane, ethanolamine, ethylamine, methylamine, isopropylamine, trimethylamine). Screening tests were conducted outdoors in natural screwworm fly habitats with the Bishopp-type fly trap presently being used in research and eradication programs. Twenty-eight of these traps were hung in a north-south line 500 m long along the edge of a dense growth of small trees and shrubs that bordered a drainage ditch near the laboratory and the screwworm rearing plant. Sterile flies that escaped from the packaging and loading operations and those released daily in quality control agility tests for the eradication program assured a rather high and stable, though sterile, fly population.

Preliminary tests were made to find a satisfactory method of exposing the chemical baits. First, we attempted to minimize chemical incompatibility. Thus 10 μ l of each of the 6 amine compounds (last 6 chemicals listed) were combined in 10 ml of glycerol, and 10 μ l of each of the remaining 24 chemicals (except 10 mg of the crystalline compounds—benzoic acid, indole and skatole) were combined in 10 ml of glycerol. The glycerol solutions were in separate 10-cm-diam open dishes and placed in bait pans hung beneath the traps. Small but consistent catches of flies prompted us to increase the amount of each chemical per trap to 500 μ l or 500 mg. Also the 24 non-amines were divided into 2 groups, the acids and aldehydes (the 1st 10 chemicals listed) and the alcohols and ketones (the next 14 chemicals listed).

To improve volatilization, ethanol was substituted for glycerol as a solvent. The ethanol solutions were placed in bottles containing wicks. Several trials with various kinds of wicks in combination with different quantities of ethanol indicated that bottles containing 40–50 ml of ethanol and a No. 2 dental roll extending 1.3 cm above the mouth of the bottle provided a steady, prolonged release of the volatile material.

¹ Diptera: Calliphoridae.

² Received for publication Dec. 22, 1975.

³ Screwworm Research Lab., Agric. Res. Serv., USDA, Mission, TX 78572. Retired Dec. 28, 1974. Present address: Route 6, Box 237-D, Fort Payne, AL 35967.

⁴ Vet. Toxicol. and Entomol. Res. Lab., Agric. Res. Serv., USDA, College Station, TX 77840.

⁵ Screwworm Research Lab., Agric. Res. Serv., USDA, Mission, TX 78572.

⁶ Star Route, Box 371, Elizabeth, CO 80107.

⁷ We thank the following technicians at the Screwworm Research Laboratory for their help in conducting this study: Erasmo Lopez and Francisco C. Gonzalez for trapping, and Brenda K. Anthony for sorting and identifying the screwworm flies.

O. H. Abraham

When the 3 groups of chemicals (amines, acids and aldehydes, and alcohols and ketones) were tested separately, the 1st and 2nd groups were very weakly attractive and the 3rd group was moderately attractive to screwworm flies. When combined, groups 2 and 3 complemented each other, but the 3rd group (amines) used in combination with either or both of the other 2 groups markedly decreased the attractiveness of the bait. No further studies were conducted with the amines.

Subsequently, we found that more screwworm flies were trapped when the chemicals were separated from each other by placing a few milliliters without ethanol and wick in a vial and grouping the vials in a bait pan. This method of exposure also simplified the evaluation of single compounds.

Finally, in an effort to categorize the remaining 25 chemicals as attractive, neutral or repellent, we omitted one or a small group of chemicals in tests. A resulting decrease of at least 10% in the number of screwworm flies trapped indicated that the omitted chemical was beneficial; little or no difference in catch suggested that the chemical was neither helpful nor harmful; a larger catch indicated that the omitted chemical was repellent. Whenever a repellent chemical was identified, it was eliminated from further testing, and the remaining mixture was designated the new standard for further testing. Tests were repeated when results were questionable. Although liver was almost always included in a test, the results of each treatment also were compared with the newest chemical standard.

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Cytogenetics of a Local Population of the Screwworm, *Cochliomyia hominivorax*,¹ from Northeastern Mexico²

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ABSTRACT

Ann. Entomol. Soc. Am. 74: 582-589 (1981)

Larvae of the primary screwworm, *Cochliomyia hominivorax* (Coquerel), were collected near Aldama in Tamaulipas, Mexico. 3rd-instar, crawl-off larvae from 19 single-egg masses were obtained from cattle hosts, and from 8 other single-egg masses off sheep. Of the six chromosome pairs in this species, the sex, II, V, and VI pairs were polymorphic. The small X and Y sex chromosomes had five and four variants, respectively. F₁ and F₂ larval samples from cattle and sheep differed markedly. There were more fluorescently spotted X chromosomes in cattle screwworms than in sheep, and chromosome VI was polymorphic in cattle screwworms but monomorphic in sheep. Both cattle and sheep material showed significant heterozygote deficiency from expected values and a striking pattern of X chromosome-based homotypic mating.

The eradication program against the screwworm, *Cochliomyia hominivorax* (Coquerel), succeeded first on Curaçao in 1954, then in the southeastern United States between 1957 and 1960 (Knippling 1960). With the sterile-insect release method proven worthy in the field, focus shifted to controlling screwworms in the U.S. southwest and in northern Mexico. Since 1962, screwworm populations in this region have been suppressed, although not eradicated, resulting in an estimated annual savings to the U.S. cattle industry alone of at least \$1 billion (Scruggs 1975). The sterile released flies have recently come into contact with wider environmental diversity and year-round populations in Mexico, and it becomes important to characterize the genetics of native screwworm populations to help maximize the mating compatibility of released strains. The genetics of mass-produced screwworm flies has been examined with respect to isozymes (Bush and Neck 1976, Whitten 1980) and chromosome morphology (Kaufman and Wasserman 1957). However, knowledge of genetic variation in native screwworm populations is lacking. Herein is described the cytogenetics of a local population from the northeastern Mexican state of Tamaulipas. A group of geneticists at the University of Texas, Austin, also have been looking at the cytogenetics and morphology of native screwworm flies, partly in collaboration with my laboratory.

Materials and Methods

The research area encompassed ca. 1,500 km² around the city of Aldama in Tamaulipas. Aldama, some 30 km from the Gulf of Mexico, was until recently a perennial screwworm outbreak area. Mesquite dominates the rangeland in the open range and hardwood occurs in dense thickets near the coast. Between February and June 1979, we took samples from ranch cattle and penned sheep. 3rd instars col-

lected from wounds in the field were frozen in liquid nitrogen, and fertile egg masses were reared in a field laboratory until 3rd-instar samples could be taken. Nineteen cattle strains were collected from six different locations in the Aldama area between the periods of 15 February 1979 and 22 June 1979. The eight sheep-derived strains came from four animal pen sites between the 10 February 1979 and 18 May 1979. All samples were transported to our Mission, Tex., facility.

We prepared larvae by the technique of John Ellison (unpublished data).³ Each larval brain was dissected into hypotonic distilled water for 5 min, fixed in 45% acetic acid for 2 min, then squashed firmly under a cover slip. The slide was frozen in liquid nitrogen to facilitate removing the cover slip. Dehydration in 95% EtOH for 5 min was followed by RNA digestion with ribonuclease (RNase) A (Sigma Co., no. R-5000) for 15 min. Finally, the remaining nuclear tissue was stained in a dilute (ca. 0.1%) aqueous solution of quinacrine dihydrochloride (Sigma Co., no. Q-0250) for 1 min and mounted in a buffering, preserving solution of 0.05 M sodium cacodylate, 3% formaldehyde, and 50% sucrose. Prepared slides were examined with ultraviolet (UV) light microscopy to reveal quinacrine fluorescing regions of mitotic chromosomes. Complete chromosome information from a minimum of five cells per individual and five individuals (including both sexes) per strain was considered adequate. Desirable preparations were photographed; chromosomes were measured from enlarged prints, and idiograms were prepared. Analysis of the larval F₁ karyotypes allowed inferences to be made as to the karyotypes of both wild parents involved in each cross. From the individual chromosome frequencies, random mating karyotype expectations may be computed, and the latter compared statistically to the karyotype frequencies actually observed.

¹ Diptera: Calliphoridae

² Received for publication 2 March 1981.

³ John R. Ellison, Dept. of Zoology, Univ. of Texas, Austin.

The relatedness of screwworm strains may be indicated by the similarities of their respective karyotypes. Such a measure can be defined simply as the number of polymorphic chromosomes held in common by two specific strains divided by the total number of polymorphic chromosome types in those two strains. Starting with a single cattle or sheep strain, such a similarity index may be computed for all remaining cattle and sheep strain comparisons. As a result, each cattle strain will have a set of indices for 18 other cattle strains plus eight sheep strains, whereas each sheep group will have indices for seven remaining sheep strains and all 19 cattle strains.

Samples for comparative reasons were prepared as described above for some of these Aldama strains as well as other *Cochliomyia hominivorax* and *C. macellaria* (F.), secondary screwworm, strains maintained at the Screwworm Research laboratory in Mission, Tex. Two different sterile fly strains were aerially released over the study area during 1979—the 009 strain until March, followed by the Aricruz strain. Both were monitored through periodic sampling of factory-produced larvae.

Results

Twenty-seven different single-egg mass lines from Aldama were analyzed. Of these, 14 are F_1 samples—all eight of those derived from sheep, and six of those from cattle. The remaining 13 cattle-derived strains, analyzed from F_2 samples from the Mission laboratory, are included because the karyotypes of the native parents could still be determined almost completely.

C. hominivorax, including material from various areas of the United States and Latin America, has six pairs of chromosomes (Kaufman and Wasserman 1957, Boyes 1967, Boyes and Shewell 1975). The shortest pair are the X and Y chromosomes (males heterogametic). Figures 1 and 2 show metaphase configurations for two samples from cattle. In general, the five pairs of autosomes are somatically associated in such preparations. All five X chromosome variants detected so far (Fig. 3) were found in the Aldama samples (Table 1). Each X chromosome was acrocentric, and the shorter arm fluoresced brightly in three of the five variants. The medium-length X chromosomes were 2 to 3 μm long in metaphase configuration, whereas the longer morphs were ca. 3.5 to 4.0 μm long. The long arms each possess one or two secondary constrictions, dark bands on the fluorescing chromosomes.

Cattle samples differed from sheep samples in the frequencies of these X chromosomes. Most of the X chromosomes (87%) from cattle samples had fluorescent tips, whereas only 52% of those from sheep were so marked. The cattle and sheep samples each had a variant not found in the material from the other host (Table 1). A homogeneity chi-square test revealed a significant difference between cattle and sheep for X chromosome variants ($\chi^2_{df=4} = 17.75$, $P < 0.01$). In samples from both hosts, medium-length X chromosomes were the most frequent

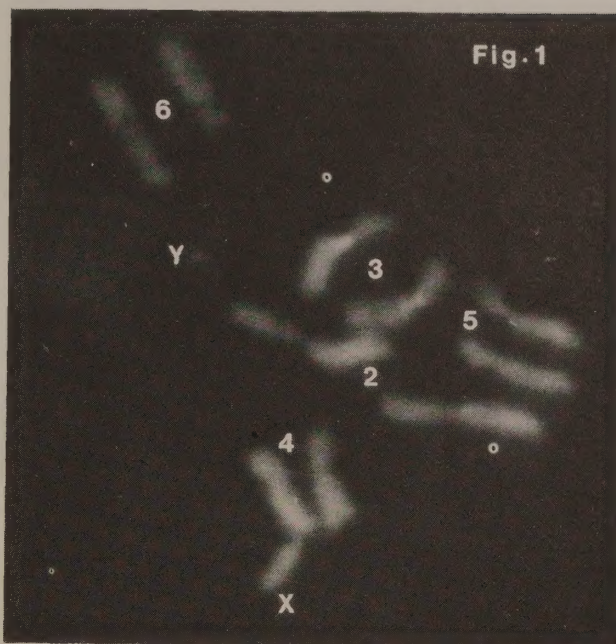


FIG. 1.—Brain metaphase preparation from F_1 male 3rd instar of *C. hominivorax* collected off cattle in Aldama, Mexico. Note medium, nonfluorescent X, very acrocentric or telocentric Y, and heterozygous long plus short VI chromosomes. The centromeric region of chromosome III fluoresces brightly with quinacrine stain.

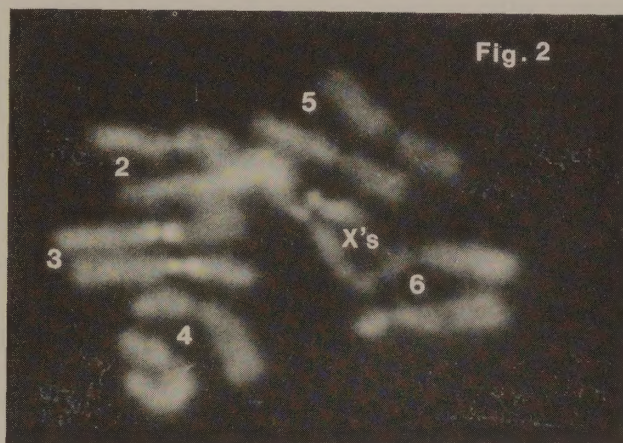


FIG. 2.—Brain metaphase preparation from F_1 female 3rd instar of *C. hominivorax* collected off cattle in Aldama, Mex. Note heterozygous long, two-banded and medium, fluorescent X's, and fluorescent spots on chromosome III.

types, fluorescent in cattle and nonfluorescent in sheep.

Four nonfluorescently tipped Y chromosomes occurred in Aldama (Fig. 4). The medium-length (1.5 to 2- μm), submetacentric form predominated in both cattle and sheep material, though the sheep material was scanty (seven values). The frequencies of the various Y morphs for cattle and for sheep did not differ ($\chi^2_{df=3} = 1.97$, $0.5 < P < 0.75$).

Of the five pairs of autosomes, the relatively long chromosome II had two variants (Fig. 5), both ca. 9 μm long in metaphase; these and the equally long submetacentric form of chromosome V were the

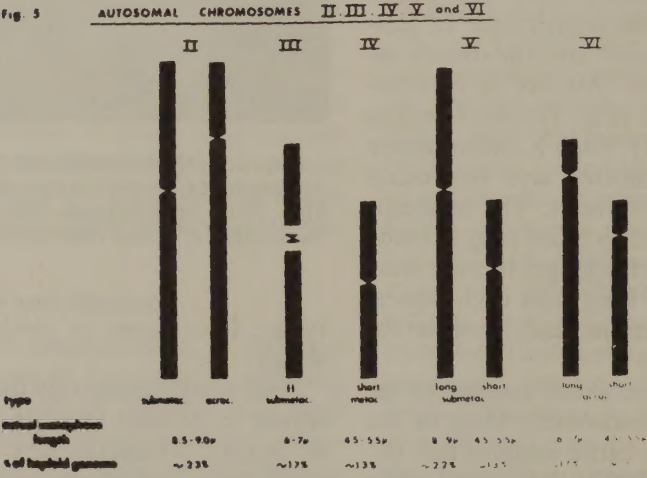
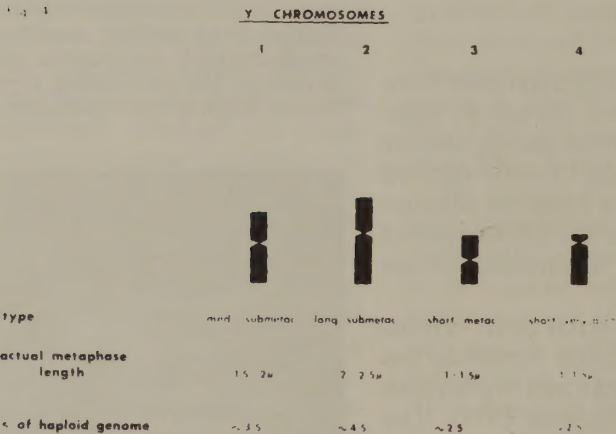
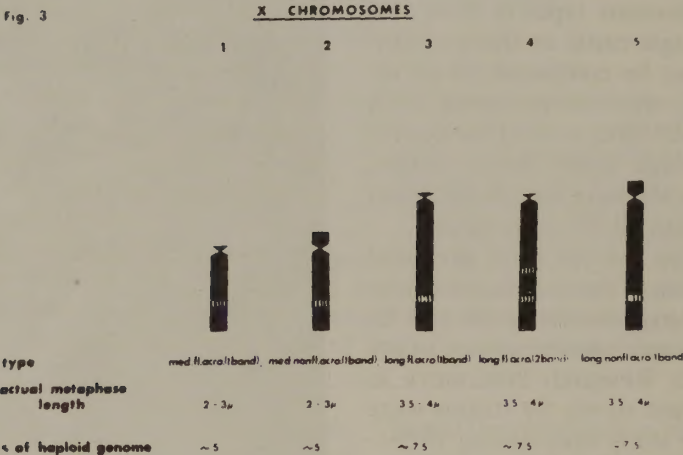


FIG. 3.—X chromosome variants found in 3rd-instar samples of *C. hominivorax* collected in Aldama, Mexico.

FIG. 4.—Y chromosome variants found in 3rd instar samples of *C. hominivorax* collected in Aldama, Mexico.

FIG. 5.—Autosomal chromosomes II, III, IV, V, and VI found in Aldama *C. hominivorax* 3rd instars. Two variants are shown for each of the II, V, and VI chromosome pairs.

longest chromosomes. The nonspotted submetacentric form comprised all but one of each of the 76 cattle-derived native fly chromosomes and 32 sheep-derived fly chromosomes (Table 1). The acrocentric type, therefore, was present just once for each host in a single heterozygous parent.

Only a submetacentric form of chromosome III was found. It was 6 to 7 μm long and, with a brightly fluorescing spot on each side of the centromere, differed strikingly from other autosomes (Fig. 3). Like chromosome III, IV had only a single morph; it was short, (4.5 to 5.5 μm long) and metacentric (Fig. 3).

Both chromosome-V polymorphisms occurred in cattle samples, but only the short variety was found in sheep material (Table 1). The longer morph was about the same length as chromosome II, and the shorter, submetacentric one was ca. 4.5 to 5.5 μm long. A test of homogeneity revealed no significant difference between cattle and sheep for the frequencies of chromosome V variants ($\chi^2_{df=1} = 1.97$, $0.10 < P < 0.25$).

The longer of the two morphs of chromosome VI occurred only among cattle samples (Table 1). A homogeneity test yields a highly significant value ($\chi^2_{df=1} = 15.5$, $P < 0.01$). Both forms were highly acrocentric; the longer morph's arm ratio was ca. 5:1, and that of the shorter was ca. 3:1. Analysis of the progeny from field collected eggs off cattle and sheep allowed inferences about the karyotypes of the native parents. As mentioned, Aldama samples are homozygous for the metacentric III and the short metacentric IV forms. Where F_1 material was involved (all eight strains from sheep and 6 of 19 strains from cattle) the genotypes of the parents could be directly inferred. For the remaining F_2 material, indirect evidence yielded nearly complete information. Where two variants of a particular chromosome (X, II, V, or VI) were present in a sample group, no conclusion could be drawn except in the case of two

different X chromosomes classed by the genotype of male F_1 progeny.

The average index of karyotypic similarity of a cattle strain compared with other cattle strains was 0.53 ± 0.023 , whereas the average for a cattle strain compared with sheep was 0.44 ± 0.023 . The difference in the average similarity indices was highly significant ($t_{df=36} = 2.752$, $P < 0.01$) for the two-tailed test. The average similarity of a sheep strain compared with cattle strains was 0.44 ± 0.032 , whereas that of a sheep strain compared with other sheep strains was 0.48 ± 0.02 , a difference not significant ($t_{df=14} = 1.05$, $P < 0.20$) for a two-tailed test. There was no correlation between karyotype similarity and the geographic distances among collecting sites.

By using 2×2 contingency chi-square tests, no statistically significant deviations from expected values were obtained for the pairwise associations of the polymorphic chromosomes (X, Y, II, V, and VI). Similarly, in $2 \times C$ chi-square tests of three or more monhomologous chromosome types, no significant association was detected. However, there was a pattern of homotypic mating based upon X chromosome type. Since X chromosome frequencies from cattle did not differ significantly between male and female parents, frequencies were pooled to determine expected numbers of matings of the particular types. Homotypic X chromosome matings involved 12 of the 19 matings yielding cattle samples and 3 of 6 sheep samples (2 of the sheep samples are incomplete for the male parent). Only 3.5 homotypic matings were expected in the cattle material ($\chi^2_1 = 25.41$, $P < 0.001$). Small sample size precludes a statistical statement about the sheep data.

Unlike the autosomes, X chromosomes may be assigned to either male or female parent when heterozygotes occur in the F_1 progeny. Accordingly, X chromosome types of male and female parents were compared in 2×2 contingency chi square tests

Table 1.—Combined frequencies of variant forms of polymorphic chromosomes of screwworms from single-egg masses taken from cattle and sheep hosts

Polymorphic chromosome	Form	Cattle		Sheep	
		Frequency	Proportion	Frequency	Proportion
X	Medium length, fluorescent, 1 band	32	0.56	7	0.32
	Medium length, nonfluorescent, 1 band	8	0.14	8	0.36
	Long, fluorescent, 1 band	8	0.14	3	0.14
	Long, fluorescent, 2 bands	9	0.16	—	—
	Long, nonfluorescent, 1 band	—	—	4	0.18
Y	Medium length, submetacentric	16	0.80	3	0.57
	Long, submetacentric	2	0.10	2	0.29
	Short, metacentric	1	0.05	1	0.14
	Short, very acrocentric	1	0.05	—	—
2	Submetacentric	75	0.99	31	0.96
	Acrocentric	1	0.01	1	0.04
5	Long	4	0.05	—	—
	Short	72	0.95	32	1.00
6	Long	28	0.37	—	—
	Short	48	0.63	32	1.00

(cattle and sheep data combined). Because of the interesting result that all X chromosome heterozygotes carried the common medium length, fluorescently tipped type, a highly significant association arose from comparing hemizygous males with females having at least one X of this kind ($\chi^2_{df=1} = 14.06, P < 0.001$). In this case, all data could be used, including the F_2 results. For the medium length, nonfluorescently spotted X and both long, fluorescently tipped X chromosomes, highly significant homotypic mating was indicated between hemizygous males and homozygous females. This appeared in spite of relatively small sample sizes. In the case of the long, nonfluorescently spotted X, insufficient data were available.

A striking pattern of excess homozygotes and a reduced number of heterozygotes was evident from both cattle and sheep samples (Table 2). Because several cattle samples were analyzed in the F_2 generation, some ambiguity arose in the assignment of karyotype to native adults for cases where polymorphism occurred. This ambiguity is reflected in

Table 2 as a range between upper and lower possible values. By using the frequencies (Table 1) for the polymorphic chromosomes (X, Y, II, V, and VI) expected frequencies of homozygotes and heterozygotes were computed upon the Hardy-Weinberg conditions of random mating in an equilibrium population having no mutation, migration, or natural selection; it was also assumed, perhaps unjustifiably, that no native male or female parent was involved in more than one of the 19 strain matings. If true, then of 19 native females ovipositing on cattle, 13 to 18 were homozygous for the X chromosome where less than 7 (6.8) homozygotes were expected. This difference was highly significant even with 13 as the actual number observed ($\chi^2_{df=1} = 8.80, P < 0.01$). A similar pattern occurred in the few sheep samples—six of eight native female adults were homozygous, but only two (2.1) were expected ($\chi^2_{df=1} = 9.81, P < 0.01$). Interestingly, the three definite heterozygotes and six other possible heterozygotes for the X chromosome in combined cattle and sheep samples all involved the medium length, fluorescent

Table 2.—Numbers of definite and possible genotypes of polymorphic chromosomes from screwworm larvae samples collected off cattle and sheep hosts^a

Host	No. of native adults	X and Y chromosome ^b types						II Chromosome types	V Chromosome types	VI Chromosome types							
Cattle	38	♀♀ X															
			1	2	3	4	5		1	2		1	2				
		1	7-12 (6-10)	1-2 (2)	0-2	0-3	0	1	1	37	1	1	0-1	2-4	1	6-12	4-16
		2	(2-4)	2	0	0	0	2	2	—	0	2	—	34-35	2	—	16-22
		♂♂ X	3	(2-4)		2	0	0	3	♀♀ X							
		4	(1-3)		(1)	2 (0-1)	0	4									
		5					0	5									
			1	2	3	4											
		Sheep ^c	16	♂♂ Y ♀♀													
					1	2	3	4	5		1	2		1	2		
1	2 (1)			1 (1)	1	0	0	1	1	15	1	1	0	0	1	0	0
2	(1)			2 (1)	0	0	0	2	2	—	0	2	—	16	2	—	16
♂♂ X	3					0	0	3	♀♀ X								
4						0	0	4									
5							2	5									
	1			2	3	4											

^a Same as those listed in Table 1.
^b Male data with nonzero value are in parentheses; zero values are left blank.
^c No sex chromosome information is available for four of eight male parents off sheep.

type. For this X chromosome type alone, homozygotes were in excess, but not significantly so unless three or more of the six uncertain genotypes were indeed homozygotes ($P = 0.05$ level).

For chromosome II, one definite heterozygote was observed for both cattle and sheep material, whereas the parental genotypes were otherwise homozygous for the submetacentric, nonfluorescent form. Similarly, chromosome V showed no homozygote excess over that expected, since two to four heterozygotes involving the rare long form were noted in cattle material. Due to ambiguity arising from F_2 samples, the number of homozygotes for chromosome VI ranged from 22 to 34, with ca. 20 (20.25) expected. Statistical significance was reached ($P \leq 0.05$) with 27 or more homozygotes in the 38 native adults. As previously noted, chromosomes V and VI were completely homozygous in sheep samples for their respective short morphs.

Of the sterile release strains, the 009 was homozygous for the medium, nonfluorescent X chromosome, the submetacentric II and III chromosomes, the metacentric IV, and short V and VI chromosomes. The Aracruz strain was autosomally identical to the 009 strain, but the X chromosomes differed. Aracruz possessed mostly the medium, fluorescent X type, and some of the long, two-banded fluorescent morph. Egg mass sterility of these strains realized at the same animal pen sites where viable forms were collected, increased from ca. 0 to ca. 60% when the strain was changed from 009 to Aracruz (Coppedge 1980).

Discussion

The results clearly show that chromosomal polymorphism is present in the Aldama population. Other variants, not found in Aldama, have been noted elsewhere. These include a relatively long, fluorescent Y chromosome which looks very much like an X chromosome, a fluorescent II, a metacentric III, and a long, submetacentric IV (McInnis, unpublished data). What is the nature of this observed variation? Based upon sample frequencies of the various morphs, expected Hardy-Weinberg karyotypic frequencies can be computed, and these can be compared with corresponding observed values. If no statistically significant differences are noted in a population at equilibrium, then the variation remains simply polymorphism. If significant differences exist, these may reflect, partially or wholly, mere accidents of sampling. Therefore, the degree of the difference must be weighed against the quality of the sample before a conclusion can be drawn. Heterozygote deficiency may reflect absence of mating solely due to geographic isolation among allopatric populations. Should the sample area encompass several such heterogenous panmictic units, then overall heterozygote deficiency will exhibit the Wahlund effect (Wahlund 1928). Care must be taken to analyze the data in the light of this potential area effect. Finally, chromosomal heterozygote deficiency may result from real behavioral or morpho-

logical differences among homozygotes leading to a corresponding level of positive assortative mating.

In the Aldama population significant differences occurred between cattle- and sheep-derived samples for frequencies of the X and chromosome VI variants (Tables 1 and 2). With the exception of one sheep pen sample collected at an outlying site, samples from cattle and sheep were roughly evenly distributed along a N-S course in the northeastern sector of the area. As mentioned earlier, there was no overall effect of distance among locations upon the relatedness of karyotypes for either cattle or sheep material. Therefore, sample source did not appear to account for the observed differences. However, date of sample collection may have been important. Only one cattle sample was collected early enough in 1979 to overlap most sheep collection dates, whereas two of eight sheep samples barely overlapped the remaining cattle collection dates. These two sheep samples resemble those from cattle, both possessing only fluorescent X chromosomes. Furthermore, long type VI chromosomes do not appear in any of the first 10 sample collections, including all 8 sheep samples and the first 2 cattle samples. Yet this particular morph was quite common afterwards. Though seasonal changes in frequencies of chromosomal polymorphisms could occur (see Dobzhansky 1970), the sample sizes are too restricted to make a conclusive statement. Collection-data differences, therefore, may explain why the karyotypes of cattle samples are significantly more like each other than they are to sheep sample karyotypes. Finally, not precluded by the data is the possibility that the bearer of one karyotype prefers to oviposit on one kind of host rather than another. How karyotype would influence this preference (if it does) remains unknown.

Heterozygote deficiency characterizes the samples from cattle for the X chromosome and perhaps also for chromosome VI. Although sample size is small for sheep material, there is a strong indication of heterozygote deficiency for the X chromosome, whereas chromosome VI is monomorphic. The only X chromosome variant not showing significant homozygosity is the most common type—the medium-length, fluorescent, single-banded morph. Curiously enough, all X chromosome heterozygotes involved this particular X type in tandem with one of the remaining X chromosomes. Excess X chromosome homozygosity may be related to the highly significant pattern of homotypic X chromosome matings. Positive assortative mating is strongly suggested for each of the X chromosomes where sufficient data are available. Females homozygous (or heterozygous in the case of the medium length, fluorescent X) for a particular X type appear to mate much more often than expected with hemizygous males of the same type. No other chromosome pair appeared to be involved in this assortative mating scheme. However, sample size was too small to detect any significant deviations from expected values for chromosomes II and V which have rare variants.

The contingency χ^2 tests indicated that no significant association exists between or among any set of nonhomologous polymorphic chromosomes. Therefore, the chromosomal variation observed in Aldama can be classified as polymorphism with a strong pattern of homotypic mating superimposed. The X chromosome alone seems to show this pattern, in spite of a number of heterozygotes for the common variant. Continual strengthening of homotypic mating patterns with the consequent reduction of heterozygotes would, in the extreme, lead to complete mating isolation and species formation. However, there is no a priori reason to suspect that an intensification of such a mating system is occurring or is irreversible. Only a long-term study in the test area would conclusively show any directional mating trends. Of course, in this instance the eradication program could not wait when given the opportunity to free an area of screwworms in the barrier zone. But if some positive assortative mating exists among native flies, then it is possible that a similar discriminating mating system will apply when sterile flies are added.

Judging from the field performances in Aldama of the two sterile fly strains, 009 and Aricruz, the mating efficiency of sterile male flies with native females may have been, at least partly, X chromosome dependent. Based upon the only available chromosome data reported here (unfortunately collected during the release of sterile insects in Aldama) and the karyotypes of the release strains, the 009 strain was much less compatible with native screwworms than was the Aricruz strain with regard to X chromosomes. The X chromosome found in the 009 line was present at only about 20% frequency in the native population. That males of this strain failed to sterilize even one-half of this percentage of native females in Aldama may also strongly relate to the fact that the 009 strain had been mass reared for 2.5 years and perhaps had become debilitated. In addition, the 009 flies had been chilled (10 to 12°C) as pupae and shipped by refrigerated truck a long distance before release—two practices eliminated during the release of Aricruz flies. However, X chromosomes in the newly colonized Aricruz strain were at about a 60% level in the native population—roughly the same percentage of sterility attained in the field by releasing the Aricruz strain. The suppressing influence of this strain was such that by the end of the summer of 1979 Aldama was declared screwworm free.

The potential interrelatedness of chromosomes may be speculated upon by an examination of Fig. 1, 2, and 3. The five X chromosomes (Fig. 1) may be related through a series of stepwise mutations involving additions or deletions of chromatin. The presumably A:T rich fluorescently tipped forms would appear to have a more obscure relationship to similarly sized nonfluorescently tipped forms. The four Y chromosomes (Fig. 2) may be related by additions or deletions of chromatin for differences in length, and by pericentric inversions for differences

in centromeric position. Similar mechanisms could have linked various autosomal chromosomes to each other (Fig. 3).

Because the screwworm occurs widely across Central and South America and the Caribbean, reproductively isolated populations have arisen. The eradication program must concern itself with genetic differences encountered from area to area, since these directly affect released sterile insects. As the barrier zone pushes further south into Mexico, and perhaps later into Central America, areas conducive to year-round populations of screwworms will be met. Genetic analyses of natural populations such as Aldama's will be needed to prepare better the eradication program against locally adapted native strains.

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**Antifertility Effects of Benzylphenols and Benzyl-1,3-Benzodioxoles¹ on
Screwworm Flies²**

S. C. RAWLINS³, L. JURD⁴, AND J. W. SNOW⁵

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Antifertility Effects of Benzylphenols and Benzyl-1,3-Benzodioxoles¹ on Screwworm Flies²

S. C. RAWLINS³, L. JURD⁴, AND J. W. SNOW⁵

ABSTRACT

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Five compounds from structurally-modified, biologically active plant materials were evaluated for their antifertility effect on *Cochliomyia hominivorax* (Coquerel). When 0- to 5-day-old flies were treated orally with concentrations of 0.01-1.0% of three and 0.1-1.0% of two of the compounds, complete sterility of female flies was obtained, whereas only J2644 (2,4-bis (1,1-dimethylethyl)-6-(4-methoxyphenylmethyl)phenol) affected fertility of males. The sterilants did not adversely affect the insemination and fecundity rates, and only the highest dosages of J2706, (2,4-bis(1,1-dimethylethyl)-6-(1-phenylethyl)phenol), J2693 (5-(2-propenyl)-6-(methoxyphenylmethyl)-1,3-benzodioxole), and J2581 (5-ethoxy-6-(4-methoxyphenylmethyl)-1,3-benzodioxole), (1.0%) proved lethal to the flies.

J2419 (2,4-bis(1,1-dimethylethyl)-6-(phenylmethyl)phenol, or J2644 continued to be effective up to the 3rd gonotrophic cycle when most of the flies were already dead. Exposure to 0.25% of J2644 for the 1st 24 or 48 h halved or eliminated fertility, respectively. Increasing dosages by 4-fold did not significantly affect efficiency of the chemosterilants. Single meals of 1.0% J2644 or of J2419 were effective in giving complete sterility.

Antifertility effects on screwworm flies of aziridines, folic acid derivatives, anthelmintics, and various sulfonate, boron, and vanadium compounds are well documented (Crystal 1963, 1964, 1966, 1968 a,b, 1970, 1971, Settepani et al. 1969). However, compounds of these types have not been developed as practical sterilants for the screwworm, *Cochliomyia hominivorax* (Coquerel), because of their inefficiency or probable toxic and mutagenic effects on mammals. Some years ago, a program was initiated at the Western Regional Research Center to determine whether biologically active plant materials could be modified structurally to give new classes of effective, and possibly safer, insect control agents. This program led to recognition (Jurd et al. 1979) that a variety of simple benzylphenols and benzyl-1,3-benzodioxoles, which are nonmutagenic in standard Ames' bacterial tests and have low mammalian toxicity, shows significant sterilant activity against the housefly, *Musca domestica* L. However, it was not known if these new sterilants were also effective against the screwworm fly. Therefore, the sterilant properties of 5 of the more promising compounds were evaluated, and the results are reported here.

Materials and Methods

These tests were conducted on mass-reared screwworm flies (009 strain) which had been obtained as pupae from the screwworm fly plant at Mission, TX. The flies were held in cages with water and undiluted corn syrup in a colony room maintained at ca. 27°C and 60% RH with a 12-h photoperiod beginning at 6 a.m. Adults that emerged were lightly anesthetized with CO₂ the same day for ease of manipulation.

Technical grades of the following compounds were used: 2,4-bis(1,1-dimethylethyl)-6-(phenylmethyl)phenol

(Code No. J2419); 2,4-bis(1,1-dimethylethyl)-6-(4-methoxyphenylmethyl)phenol (J2644); 2,4-bis(1,1-dimethylethyl)-6-(1-phenylethyl)phenol (J2706); 5-ethoxy-6-(4-methoxyphenylmethyl)-1,3-benzodioxole (J2581); and 5-(2-propenyl)-6-(4-methoxyphenylmethyl)-1,3-benzodioxole (J2693).

Peroral Treatments

Because of low solubility of these sterilants in water, 3 different feeding methods were investigated. First, suspensions of the sterilants in an aqueous sugar medium were prepared by adding solutions of weighed quantities of the sterilants in 5 ml acetone to 10 g of sugar solution (100g/100ml). After evaporation of acetone by gentle heating, the residue was homogenized, diluted with more sugar solution to give the desired sterilant concentration, and fed to flies in Dixie® paper cups, 7 cm diam; rice hulls were placed on the surface of the food to prevent flies from adhering to the viscous mixture. Second, homogeneous suspensions of the sterilants in a corn syrup medium were prepared by gradual mixing in a mortar and pestle and were fed to flies in paper cups as already described. Third, since the sterilants are being considered for incorporation in a bait for the Screwworm Adult Suppression System (SWASS), compounds J2419 and J2644 were each formulated in a standard bait of Elmer's® glue (52%), sucrose (31%), and dried blood (17%) (Coppedge et al. 1978). Known weights of the sterilants, dissolved in 2 ml of acetone, were added to the sucrose, and the acetone was expelled by gentle heating. Elmer's glue and dried blood were then added with thorough mixing, and the solid bait was offered to the flies in paper cups.

Varying concentrations of sterilants were fed to the flies for the 1st 5 days, and the sterilant diet was then replaced with pure corn syrup. On the 7th day, 3 replicates of 10 flies were stimulated to oviposit (Crystal 1963), and those which oviposited were checked for insemination by an examination of the spermathecae for live spermatozoa. The total number of eggs per fly was estimated volumetrically, and the hatchability of these eggs (fertility) was determined 24 h later (at 37°C). Per-

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centage inhibition of oviposition or egg hatch (x) was expressed as $\%x = 100 - (\% \text{ experimental group} / \% \text{ control group}) \times 100$.

The effect of the chemosterilants on males and females was determined by treating the sexes separately with 0.25% of the compounds in sugar solution then permitting them to mate with untreated virgins. Antifecundity and antifertility effects were observed as described earlier.

The 2 most potent compounds, J2419 and J2644, were assayed for reversibility of sterilitant effect with age or in subsequent gonotrophic cycles. Four groups of 150 females and 100 males were exposed to 0.25% of the compounds or a control on days 0–4. Thereafter, flies were fed corn syrup up to day 16. Inseminated but nulliparous females were stimulated to oviposit on days 6, 13, or 16. Fertility studies were performed on these eggs. Similarly, treated females were stimulated to oviposit on day 6, and a sample of 30 females was scored for insemination and hatch of the eggs. On day 10, all parous flies were again stimulated to oviposit, and samples were scored for insemination, fecundity, and egg hatch. Those processes were repeated on day 14.

Batches of 100 males and 100 females were fed J2644 (0.25% in corn syrup) for the following periods after emergence: 24, 48, 72, 96, and 120 h. At 7 days, flies were assessed for oviposition and the fertility of the eggs to establish the minimum period and age of exposure for infertility to occur.

To assess the period of maximum susceptibility of screwworms to the chemosterilant activity, batches of 65 females and 65 males were fed 0.5% of J2419 or J2644 for 24 h on days 0–1, 3–4, or 6–7. The sterilitant diets, formulated in corn syrup, were replaced by pure corn syrup before and after the treatments except for 48-h starvation period prior to the 3–4 and 6–7 day treatments. Fertility of these flies was assessed on day 7.

Minimum dosages of J2419 and J2644 required for effective sterility of screwworm flies after 24 h exposure were assessed as follows. One-day-old flies (100 males and 100 females) were starved for 24 h and subsequently exposed for 24 h to dried blood (SWASS) diets containing 0.25, 0.5, and 1.0% wt/wt concentrations of J2419 or J2644. Flies were then given corn syrup until egg fertility was determined after the 7th day.

The effects of a single meal of J2644 and J2419 on screwworm flies were examined. A mixed population of females and male screwworm flies was starved for 24 h and then permitted to feed on concentrations of 1% J2419 or J2644 in corn syrup. When a group of ca. 15 flies was settled to feed, the cup with the flies was removed to a new cage where all flies were permitted to feed to surfeit. The process was repeated until all flies, which had fed for a mean of ca. 2 h on a sterilitant, were grouped together. This experiment was performed on days 2, 4, and 6. The controls received only corn syrup. Fertility data were obtained for the 3 groups and the controls.

Results and Discussion

Table 1 lists effects of varying concentrations of the 5 sterilitants in sucrose solutions or corn syrup on

screwworm flies. Flies tolerated 1% concentrations of J2644 and J2419 (to a lesser extent), and lower concentrations of J2581, J2693, and J2706 in sucrose with little mortality. The percentages of female flies ovipositing or inseminated were moderate to high (60–100%) in all cases except those treated with J2693. With this compound, the number of flies ovipositing dropped to as low as 30%. Similarly, the number of eggs oviposited by inseminated females was not severely depressed by any treatment except that with 0.25 and 0.5% J2693 which caused ca. 40% inhibition. In aqueous sugar at concentration between 0.25–1.0%, all compounds except J2581, totally inhibited egg hatch. However, when fed in corn syrup, J2581, like J2644 and J2693, caused sterility at 0.1%. J2706 and J2419 were even more effective; 100% sterilization was obtained with concentrations of only 0.01%. The higher level of sterility of eggs oviposited by flies which had been fed the sterilitant in corn syrup may be due to the relative slowness of digestion of the more concentrated sugar (corn syrup) and thus the availability of the sterilitant over a longer period (Crystal 1968 a). The sterilitants are chiefly effective against females. Thus, although complete sterility resulted from mating treated females with normal males (Table 2), insemination of normal females by treated males did not significantly inhibit egg hatch except in the case of male flies treated with J2644 which produced 43% sterility. The low toxicity of these sterilitants and their effectiveness against females are dissimilar to those of aziridines which are more active against males. Thus, Crystal (1971) reported that tarsal treatment of screwworm flies with N,N' -tetramethylenebis(1-Aziridinecarboxamide) led to complete sterility in the male, while oral treatments caused high mortality.

When male and female screwworms were fed J2419 or J2644 at 0.25% over the 1st 5 days of life, no recovery of normal fertility occurred up to the 16th day, at which time most of the flies were dead (Table 3). J2644 and J2419 continued to be effective in the 2nd and 3rd gonotrophic cycles. They caused 95 and 83% inhibition of egg hatch, respectively, in the 2nd and 59 and 76% inhibition, respectively, in the 3rd gonotrophic cycles, even though exposure to the chemosterilant was removed on the 5th day, 2 days before the end of the 1st gonotrophic cycle (Table 3). In contrast to the near permanent infertility produced by J2644 or J2419, eggs from screwworms treated with 10% diflubenzuron regained 97% of their fertility by the 15th day posttreatment (Crystal 1978).

Data on the minimum period and age of exposure to 0.25% of J2644 required to produce infertility were obtained. Whereas exposure for 0–24 h only caused $46.8 \pm 7.1\%$ inhibition of hatchability, after 48 h, ca. 100% sterility was obtained. Treatment with 0.25% J2644 or J2419 following 2 days starvation also was effective in giving 90–100% inhibition of egg hatch after exposure for 24 h, although massive mortality also resulted from the long starvation. There was an increase in susceptibility (as indicated by the % inhibition of egg hatch to the effects of 0.25% J2644 on days 3–4 (82.0 ± 17.6) and 6–7 (95.4 ± 3.0) over days 0–1 (3.3 ± 3.7)). However, flies treated with 0.25% J2419 the

Table 1.—Chemosterilant activity of various dosages of 5 chemicals administered perorally to screwworm flies in sucrose solution (SS) and in corn syrup (CS)^a.

Chemical	Concn	Medium	Survival %		% flies		Inhibition of ^b fecundity (%)	Inhibition of ^b egg hatch (%)
			♂	♀	Ovipositing	Inseminated		
J2706	Control	SS	92.7	92.0	92.3	96.3	0	0
		CS	50.0	81.3	83.3	100	0	0
	0.025	SS	90.7	89.3	86.7	100	21.7±2.1	43.7±4.5
	0.25		84.7	73.3	93.3	100	15.7±4.9	100
	0.50		78.0	88.0	90.0	96.3	25.5±7.2	100
	1.00		0	0				
J2419	0.01	CS	54.0	71.3	63.3	94.7	36.9±6.8	100
	0.10		47.0	68.0	90.0	100	0	100
	0.025	SS	88.0	94.7	100	100	9.5±2.1	27.8±5.1
	0.25		96.7	96.7	83.3	100	28.1±6.4	100
	0.50		84.0	94.7	93.3	100	23.3±5.9	100
	1.00		21.6	20.6	90.3	92.9	0	100
J2693	0.01	CS	46.0	49.3	66.7	100	0	95.2±8.0
	0.10		55.0	88.7	95.0	100	15.8±4.5	100
	0.025	SS	92.7	100	86.7	96.3	10.5±6.2	13.9±7.4
	0.25		88.0	86.7	30.0	58.3	40.8±12.1	100
	0.50		64.0	70.7	50.0	88.9	39.4±9.4	100
	1.00		0	0				
J2581	0.01	CS	49.0	54.0	80.0	100	39.6±13.6	13.2±10.7
	0.10	CS	45.0	92.7	65.0	100	3.9±1.7	100
	0.025	SS	100	94.7	86.7	100	10.6±6.2	15.5±9.1
	0.25		97.3	91.3	73.3	86.3	27.3±7.5	68.2±5.2
	0.50		66.7	79.3	60.0	94.4	24.2±8.3	72.4±6.3
	1.00		0	0				
J2644	0.01	CS	68.0	74.7	83.3	96.0	10.0±2.9	2.0±1.8
	0.10		35.0	88.7	55.0	100	0	99.8±7.2
	0.025	SS	94.0	94.0	86.7	100	21.5±4.9	33.4±8.2
	0.25		85.3	91.3	80.0	95.2	30.2±8.6	100
	0.50		81.3	92.0	83.3	92.1	25.9±7.9	100
	1.00		97.3	92.0	86.7	92.3	12.9±6.1	100
	0.01	CS	54.0	76.0	83.3	100	19.4±23.4	5.2±7.6
	0.10		63.0	66.0	75.0	100	0	100

^a Chemosterilants were available to the flies for the 1st 5 days of life.

^b % Inhibition = $100 - \frac{\text{experimental effect (\%)}}{\text{control effect (\%)}} \times 100 \pm \text{SD}$.

day before oviposition (days 6–7) did not produce a high proportion of infertile eggs (17.3±11.3% inhibition) despite complete sterility on days 0–1 and 3–4. Thus, in addition to the sterilant concentration level, both the time (stage of egg development) and duration of feeding

Table 2.—Antigonodotropic activity of 0.25% of 5 chemosterilants administered^a perorally to male and female screwworm flies separately.

Treatment		% flies		Mean no. eggs/female±SD	Mean % inhibition of ^c egg hatch±SD
		Ovipos- iting	Insem- inated		
♂	♀				
1 UT ^b	UT	86.7	88.4	228.0±82.1	0
2 UT	J2706 ^d	79.5	72.9	266.0±53.7	100.0
3 J2706	UT	92.6	61.2	300.1±48.8	14.0±2.5
4 UT	J2419	73.0	91.7	285.0±57.0	100.0
5 J2419	UT	90.0	51.0	324.8±49.3	4.8±2.2
6 UT	J2644	93.3	78.9	236.8±38.3	100.0
7 J2644	UT	86.2	46.1	263.3±91.5	43.3±6.7
8 UT	J2693	3.3	3.3	15.0 ^e	100.0
9 J2693	UT	85.0	24.1	199.5±75.4	0
10 UT	J2581	100.0	50.0	162.9±41.2	78.6±8.4
11 J2581	UT	90.0	42.9	222.9±30.7	14.7±3.1

^a Chemosterilants administered in sucrose solutions.

^b UT, = untreated.

^c % Inhibition = $100 - \frac{\% \text{ hatch of treated group}}{\% \text{ hatch of control group}} \times 100$.

^d Numbers are codes for the 5 chemosterilants.

^e Only one female oviposited.

^a Chemosterilant administered in corn syrup.

^b % Inhibition of embryogenesis: $100 - \frac{\text{experimental effect (\%)}}{\text{control effect (\%)}} \times 100$.

^c No survivors.

determine the degree of induced infertility. At stage 2 of ovarian development (24 h) (Adams and Reinecke 1978, Crystal 1978), 0.25% J2644 depressed egg hatchability by only 3% in contrast to the 82% sterility obtained at stage 4 (48 h). When treatment was given to flies in dried blood bait over 24 h, a 4-fold increment in dosage only produced slight increases in infertility. Thus, 0.25–1.0% of compound J2419 caused 86–99%, and 0.25–1.0% of compound J2644 caused 74–85% inhibition of egg hatch.

Table 3.—The recovery of fertility in screwworm flies after chemosterilant treatments^a.

Day of oviposition	Gonotrophic cycle	Mean % inhibition of egg hatch (±SD) of flies given 0.25% of	
		J2419	J2644
6	1	100	100
13	1	100	100
16	1	— ^c	98.7±0.9
10	2	83.4±2.6	95.2±3.8
14	3	75.6±0.9	59.4±6.0

Flies can be completely sterilized from a single meal of 1.0% J2644 if fed on day 4 (Table 4). When the sterilant was given earlier (day 2) or later (day 6), sterilization was reduced to 68–84%. Diflubenzuron has an opposite effect, increasing infertility in 6-day-old screwworm flies by 8 times that in 3-day-old flies (Crystal 1978). As observed by Crystal (1978), there seems to be a peak uptake of food (chemosterilant) on the 3rd–4th day (Table 4), compared with the 0–1st or 6–7th day. J2419 (1.0%), however, effectively sterilized the flies even when fed on the day immediately before oviposition.

Table 4.—Effects of exposure of male and female screwworm flies to 1% dosages of compounds J2644 or J2419^a for one meal only.

Chemosterilant	Day of exposure	% inhibition of egg hatch	Stage of egg development at treatment
J2644	2	68.3 ± 9.5	4.2 ± 0.4
	4	100 ± 0	4.5 ± 0.7
	6	84.3 ± 11.8	9.5 ± 1.1
J2419	2	0 ± 0	4.2 ± 0.4
	4	95.1 ± 2.9	4.5 ± 0.7
	6	100 ± 0	9.5 ± 1.1

^a Administered in corn syrup.

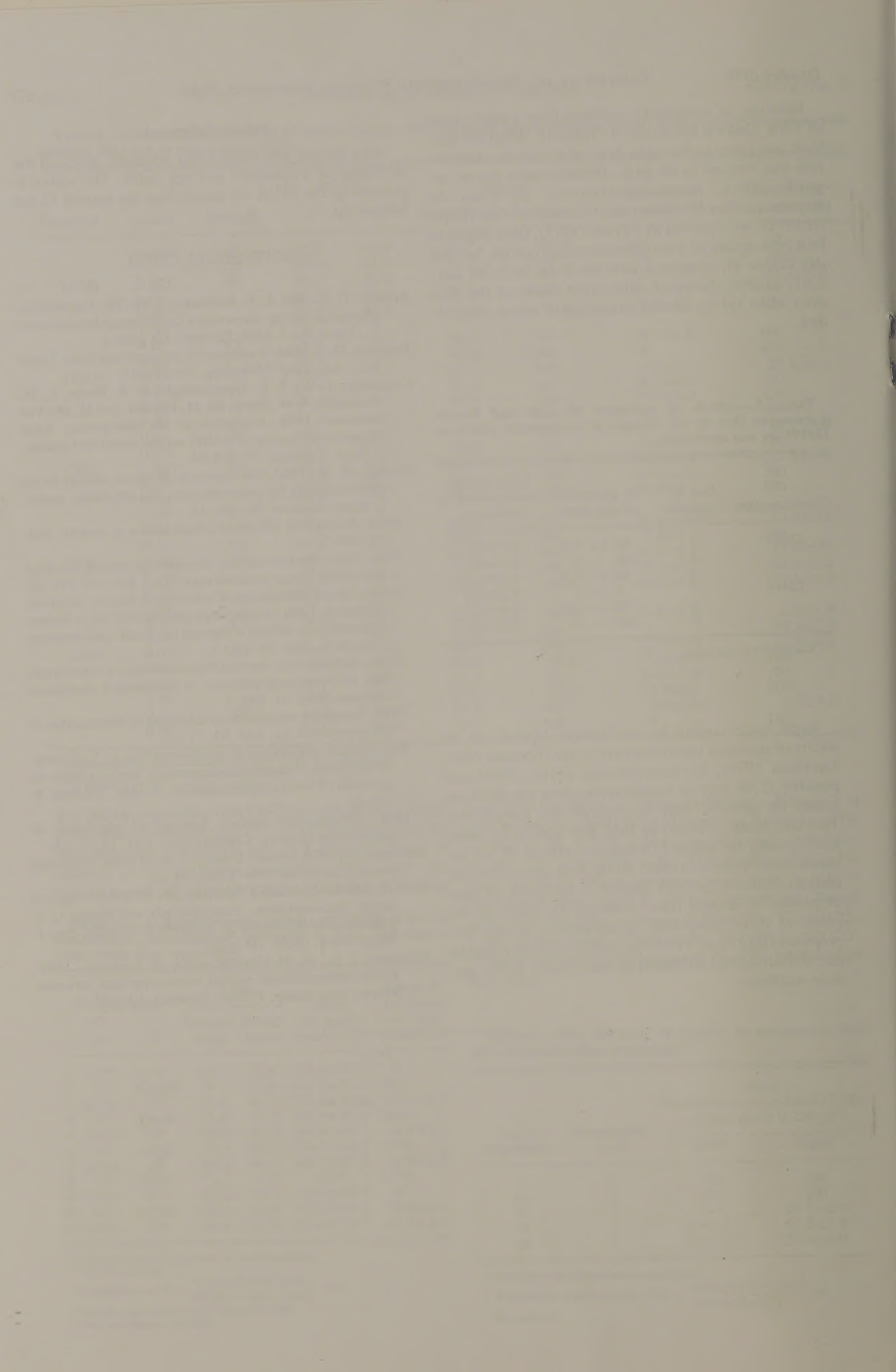
Since insect control by sterilization depends on the ability of sterilized individuals to survive (Bertram 1964, Davidson 1974), the benzylphenols J2419, J2644 and possibly J2706 may be considered to show excellent potential for possible field use against the screwworm. This conclusion is based on their low toxicity to males and females, the absence of negative effects on the aggressiveness of males (based on the high rates of insemination), and on the high degree of infertility of eggs oviposited by treated flies. Although the mode of the action of these compounds is not known, it has been proposed (Jurd et al. 1979) that sterilant activity may be due to microsomal oxidation to biologically active quinone methides.

Acknowledgment

Josie Salinas and Adan Luna diligently assessed the inhibition of oviposition and egg hatch. The author is grateful to the IAEA for sponsoring the current 12-mo fellowship.

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**Antifertility Effects of Benzylphenols and Benzyl-1,3-Benzodioxoles¹ on
Screwworm Flies²**

S. C. RAWLINS³, L. JURD⁴, AND J. W. SNOW⁵

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S. C. RAWLINS³, L. JURD⁴, AND J. W. SNOW⁵

ABSTRACT

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Five compounds from structurally-modified, biologically active plant materials were evaluated for their antifertility effect on *Cochliomyia hominivorax* (Coquerel). When 0- to 5-day-old flies were treated orally with concentrations of 0.01-1.0% of three and 0.1-1.0% of two of the compounds, complete sterility of female flies was obtained, whereas only J2644 (2,4-bis(1,1-dimethylethyl)-6-(4-methoxyphenylmethyl)phenol) affected fertility of males. The sterilants did not adversely affect the insemination and fecundity rates, and only the highest dosages of J2706, (2,4-bis(1,1-dimethylethyl)-6-(1-phenylethyl)phenol), J2693 (5-(2-propenyl)-6-(methoxyphenylmethyl)-1,3-benzodioxole), and J2581 (5-ethoxy-6-(4-methoxyphenylmethyl)-1,3-benzodioxole), (1.0%) proved lethal to the flies.

J2419 (2,4-bis(1,1-dimethylethyl)-6-(phenylmethyl)phenol, or J2644 continued to be effective up to the 3rd gonotrophic cycle when most of the flies were already dead. Exposure to 0.25% of J2644 for the 1st 24 or 48 h halved or eliminated fertility, respectively. Increasing dosages by 4-fold did not significantly affect efficiency of the chemosterilants. Single meals of 1.0% J2644 or of J2419 were effective in giving complete sterility.

Antifertility effects on screwworm flies of aziridines, folic acid derivatives, anthelmintics, and various sulfonate, boron, and vanadium compounds are well documented (Crystal 1963, 1964, 1966, 1968 a,b, 1970, 1971, Settepani et al. 1969). However, compounds of these types have not been developed as practical sterilants for the screwworm, *Cochliomyia hominivorax* (Coquerel), because of their inefficiency or probable toxic and mutagenic effects on mammals. Some years ago, a program was initiated at the Western Regional Research Center to determine whether biologically active plant materials could be modified structurally to give new classes of effective, and possibly safer, insect control agents. This program led to recognition (Jurd et al. 1979) that a variety of simple benzylphenols and benzyl-1,3-benzodioxoles, which are nonmutagenic in standard Ames' bacterial tests and have low mammalian toxicity, shows significant sterilant activity against the housefly, *Musca domestica* L. However, it was not known if these new sterilants were also effective against the screwworm fly. Therefore, the sterilant properties of 5 of the more promising compounds were evaluated, and the results are reported here.

Materials and Methods

These tests were conducted on mass-reared screwworm flies (009 strain) which had been obtained as pupae from the screwworm fly plant at Mission, TX. The flies were held in cages with water and undiluted corn syrup in a colony room maintained at ca. 27°C and 60% RH with a 12-h photoperiod beginning at 6 a.m. Adults that emerged were lightly anesthetized with CO₂ the same day for ease of manipulation.

Technical grades of the following compounds were used: 2,4-bis(1,1-dimethylethyl)-6-(phenylmethyl)phenol

(Code No. J2419); 2,4-bis(1,1-dimethylethyl)-6-(4-methoxyphenylmethyl)phenol (J2644); 2,4-bis(1,1-dimethylethyl)-6-(1-phenylethyl)phenol (J2706); 5-ethoxy-6-(4-methoxyphenylmethyl)-1,3-benzodioxole (J2581); and 5-(2-propenyl)-6-(4-methoxyphenylmethyl)-1,3-benzodioxole (J2693).

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Because of low solubility of these sterilants in water, 3 different feeding methods were investigated. First, suspensions of the sterilants in an aqueous sugar medium were prepared by adding solutions of weighed quantities of the sterilants in 5 ml acetone to 10 g of sugar solution (100g/100ml). After evaporation of acetone by gentle heating, the residue was homogenized, diluted with more sugar solution to give the desired sterilant concentration, and fed to flies in Dixie® paper cups, 7 cm diam; rice hulls were placed on the surface of the food to prevent flies from adhering to the viscous mixture. Second, homogeneous suspensions of the sterilants in a corn syrup medium were prepared by gradual mixing in a mortar and pestle and were fed to flies in paper cups as already described. Third, since the sterilants are being considered for incorporation in a bait for the Screwworm Adult Suppression System (SWASS), compounds J2419 and J2644 were each formulated in a standard bait of Elmer's® glue (52%), sucrose (31%), and dried blood (17%) (Coppedge et al. 1978). Known weights of the sterilants, dissolved in 2 ml of acetone, were added to the sucrose, and the acetone was expelled by gentle heating. Elmer's glue and dried blood were then added with thorough mixing, and the solid bait was offered to the flies in paper cups.

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Batches of 100 males and 100 females were fed J2644 (0.25% in corn syrup) for the following periods after emergence: 24, 48, 72, 96, and 120 h. At 7 days, flies were assessed for oviposition and the fertility of the eggs to establish the minimum period and age of exposure for infertility to occur.

To assess the period of maximum susceptibility of screwworms to the chemosterilant activity, batches of 65 females and 65 males were fed 0.5% of J2419 or J2644 for 24 h on days 0–1, 3–4, or 6–7. The sterility diets, formulated in corn syrup, were replaced by pure corn syrup before and after the treatments except for 48-h starvation period prior to the 3–4 and 6–7 day treatments. Fertility of these flies was assessed on day 7.

Minimum dosages of J2419 and J2644 required for effective sterility of screwworm flies after 24 h exposure were assessed as follows. One-day-old flies (100 males and 100 females) were starved for 24 h and subsequently exposed for 24 h to dried blood (SWASS) diets containing 0.25, 0.5, and 1.0% wt/wt concentrations of J2419 or J2644. Flies were then given corn syrup until egg fertility was determined after the 7th day.

The effects of a single meal of J2644 and J2419 on screwworm flies were examined. A mixed population of females and male screwworm flies was starved for 24 h and then permitted to feed on concentrations of 1% J2419 or J2644 in corn syrup. When a group of ca. 15 flies was settled to feed, the cup with the flies was removed to a new cage where all flies were permitted to feed to surfeit. The process was repeated until all flies, which had fed for a mean of ca. 2 h on a sterility diet, were grouped together. This experiment was performed on days 2, 4, and 6. The controls received only corn syrup. Fertility data were obtained for the 3 groups and the controls.

Results and Discussion

Table 1 lists effects of varying concentrations of the 5 sterility diets in sucrose solutions or corn syrup on

screwworm flies. Flies tolerated 1% concentrations of J2644 and J2419 (to a lesser extent), and lower concentrations of J2581, J2693, and J2706 in sucrose with little mortality. The percentages of female flies ovipositing or inseminated were moderate to high (60–100%) in all cases except those treated with J2693. With this compound, the number of flies ovipositing dropped to as low as 30%. Similarly, the number of eggs oviposited by inseminated females was not severely depressed by any treatment except that with 0.25 and 0.5% J2693 which caused ca. 40% inhibition. In aqueous sugar at concentration between 0.25–1.0%, all compounds except J2581, totally inhibited egg hatch. However, when fed in corn syrup, J2581, like J2644 and J2693, caused sterility at 0.1%. J2706 and J2419 were even more effective; 100% sterilization was obtained with concentrations of only 0.01%. The higher level of sterility of eggs oviposited by flies which had been fed the sterility diet in corn syrup may be due to the relative slowness of digestion of the more concentrated sugar (corn syrup) and thus the availability of the sterility diet over a longer period (Crystal 1968a). The sterility diets are chiefly effective against females. Thus, although complete sterility resulted from mating treated females with normal males (Table 2), insemination of normal females by treated males did not significantly inhibit egg hatch except in the case of male flies treated with J2644 which produced 43% sterility. The low toxicity of these sterility diets and their effectiveness against females are dissimilar to those of aziridines which are more active against males. Thus, Crystal (1971) reported that tarsal treatment of screwworm flies with N,N^1 -tetramethylenebis(1-Aziridinecarboxamide) led to complete sterility in the male, while oral treatments caused high mortality.

When male and female screwworms were fed J2419 or J2644 at 0.25% over the 1st 5 days of life, no recovery of normal fertility occurred up to the 16th day, at which time most of the flies were dead (Table 3). J2644 and J2419 continued to be effective in the 2nd and 3rd gonotrophic cycles. They caused 95 and 83% inhibition of egg hatch, respectively, in the 2nd and 59 and 76% inhibition, respectively, in the 3rd gonotrophic cycles, even though exposure to the chemosterilant was removed on the 5th day, 2 days before the end of the 1st gonotrophic cycle (Table 3). In contrast to the near permanent infertility produced by J2644 or J2419, eggs from screwworms treated with 10% diflubenzuron regained 97% of their fertility by the 15th day posttreatment (Crystal 1978).

Data on the minimum period and age of exposure to 0.25% of J2644 required to produce infertility were obtained. Whereas exposure for 0–24 h only caused 46.8±7.1% inhibition of hatchability, after 48 h, ca. 100% sterility was obtained. Treatment with 0.25% J2644 or J2419 following 2 days starvation also was effective in giving 90–100% inhibition of egg hatch after exposure for 24 h, although massive mortality also resulted from the long starvation. There was an increase in susceptibility (as indicated by the % inhibition of egg hatch to the effects of 0.25% J2644 on days 3–4 (82.0±17.6) and 6–7 (95.4±3.0) over days 0–1 (3.3±3.7). However, flies treated with 0.25% J2419 the

Table 1.—Chemosterilant activity of various dosages of 5 chemicals administered perorally to screwworm flies in sucrose solution (SS) and in corn syrup (CS)^a.

Chemical	Concn	Medium	Survival %		% flies		Inhibition of fecundity (%)	Inhibition of egg hatch (%)
			♂	♀	Ovipositing	Inseminated		
J2706	Control	SS	92.7	92.0	92.3	96.3	0	0
		CS	50.0	81.3	83.3	100	0	0
	0.025	SS	90.7	89.3	86.7	100	21.7±2.1	43.7±4.5
	0.25		84.7	73.3	93.3	100	15.7±4.9	100
	0.50		78.0	88.0	90.0	96.3	25.5±7.2	100
	1.00		0	0				
J2419	0.01	CS	54.0	71.3	63.3	94.7	36.9±6.8	100
	0.10		47.0	68.0	90.0	100	0	100
	0.025	SS	88.0	94.7	100	100	9.5±2.1	27.8±5.1
	0.25		96.7	96.7	83.3	100	28.1±6.4	100
	0.50		84.0	94.7	93.3	100	23.3±5.9	100
	1.00		21.6	20.6	90.3	92.9	0	100
J2693	0.01	CS	46.0	49.3	66.7	100	0	95.2±8.0
	0.10		55.0	88.7	95.0	100	15.8±4.5	100
	0.025	SS	92.7	100	86.7	96.3	10.5±6.2	13.9±7.4
	0.25		88.0	86.7	30.0	58.3	40.8±12.1	100
	0.50		64.0	70.7	50.0	88.9	39.4±9.4	100
	1.00		0	0				
J2581	0.01	CS	49.0	54.0	80.0	100	39.6±13.6	13.2±10.7
	0.10	CS	45.0	92.7	65.0	100	3.9±1.7	100
	0.025	SS	100	94.7	86.7	100	10.6±6.2	15.5±9.1
	0.25		97.3	91.3	73.3	86.3	27.3±7.5	68.2±5.2
	0.50		66.7	79.3	60.0	94.4	24.2±8.3	72.4±6.3
	1.00		0	0				
J2644	0.01	CS	68.0	74.7	83.3	96.0	10.0±2.9	2.0±1.8
	0.10		35.0	88.7	55.0	100	0	99.8±7.2
	0.025	SS	94.0	94.0	86.7	100	21.5±4.9	33.4±8.2
	0.25		85.3	91.3	80.0	95.2	30.2±8.6	100
	0.50		81.3	92.0	83.3	92.1	25.9±7.9	100
	1.00		97.3	92.0	86.7	92.3	12.9±6.1	100
	0.01	CS	54.0	76.0	83.3	100	19.4±23.4	5.2±7.6
	0.10		63.0	66.0	75.0	100	0	100

^a Chemosterilants were available to the flies for the 1st 5 days of life.

^b % Inhibition = $100 - \frac{\text{experimental effect (\%)}}{\text{control effect (\%)}} \times 100 \pm \text{SD}$.

day before oviposition (days 6–7) did not produce a high proportion of infertile eggs (17.3±11.3% inhibition) despite complete sterility on days 0–1 and 3–4. Thus, in addition to the sterilant concentration level, both the time (stage of egg development) and duration of feeding

Table 2.—Antigonodotropic activity of 0.25% of 5 chemosterilants administered^a perorally to male and female screwworm flies separately.

Treatment		% flies		Mean no. eggs/female±SD	Mean % inhibition of egg hatch±SD
		Ovipos-iting	Insem-inated		
♂	♀				
1 UT ^b	UT	86.7	88.4	228.0±82.1	0
2 UT	J2706 ^d	79.5	72.9	266.0±53.7	100.0
3 J2706	UT	92.6	61.2	300.1±48.8	14.0±2.5
4 UT	J2419	73.0	91.7	285.0±57.0	100.0
5 J2419	UT	90.0	51.0	324.8±49.3	4.8±2.2
6 UT	J2644	93.3	78.9	236.8±38.3	100.0
7 J2644	UT	86.2	46.1	263.3±91.5	43.3±6.7
8 UT	J2693	3.3	3.3	15.0 ^e	100.0
9 J2693	UT	85.0	24.1	199.5±75.4	0
10 UT	J2581	100.0	50.0	162.9±41.2	78.6±8.4
11 J2581	UT	90.0	42.9	222.9±30.7	14.7±3.1

^a Chemosterilants administered in sucrose solutions.

^b UT, = untreated.

^c % Inhibition = $100 - \frac{\% \text{ hatch of treated group}}{\% \text{ hatch of control group}} \times 100$.

^d Numbers are codes for the 5 chemosterilants.

^e Only one female oviposited.

determine the degree of induced infertility. At stage 2 of ovarian development (24 h) (Adams and Reinecke 1978, Crystal 1978), 0.25% J2644 depressed egg hatchability by only 3% in contrast to the 82% sterility obtained at stage 4 (48 h). When treatment was given to flies in dried blood bait over 24 h, a 4-fold increment in dosage only produced slight increases in infertility. Thus, 0.25–1.0% of compound J2419 caused 86–99%, and 0.25–1.0% of compound J2644 caused 74–85% inhibition of egg hatch.

Table 3.—The recovery of fertility in screwworm flies after chemosterilant treatments^a.

Day of oviposition	Gonotrophic cycle	Mean % inhibition of egg hatch (±SD) of flies given 0.25% of	
		J2419	J2644
6	1	100	100
13	1	100	100
16	1	— ^c	98.7±0.9
10	2	83.4±2.6	95.2±3.8
14	3	75.6±0.9	59.4±6.0

^a Chemosterilant administered in corn syrup.

^b % Inhibition of embryogenesis: $100 - \frac{\text{experimental effect (\%)}}{\text{control effect (\%)}} \times 100$.

^c No survivors.

Flies can be completely sterilized from a single meal of 1.0% J2644 if fed on day 4 (Table 4). When the sterilant was given earlier (day 2) or later (day 6), sterilization was reduced to 68–84%. Diflubenzuron has an opposite effect, increasing infertility in 6-day-old screwworm flies by 8 times that in 3-day-old flies (Crystal 1978). As observed by Crystal (1978), there seems to be a peak uptake of food (chemosterilant) on the 3rd–4th day (Table 4), compared with the 0–1st or 6–7th day. J2419 (1.0%), however, effectively sterilized the flies even when fed on the day immediately before oviposition.

Table 4.—Effects of exposure of male and female screwworm flies to 1% dosages of compounds J2644 or J2419^a for one meal only.

Chemosterilant	Day of exposure	% inhibition of egg hatch	Stage of egg development at treatment
J2644	2	68.3 ± 9.5	4.2 ± 0.4
	4	100 ± 0	4.5 ± 0.7
	6	84.3 ± 11.8	9.5 ± 1.1
J2419	2	0 ± 0	4.2 ± 0.4
	4	95.1 ± 2.9	4.5 ± 0.7
	6	100 ± 0	9.5 ± 1.1

^a Administered in corn syrup.

Since insect control by sterilization depends on the ability of sterilized individuals to survive (Bertram 1964, Davidson 1974), the benzyphenols J2419, J2644 and possibly J2706 may be considered to show excellent potential for possible field use against the screwworm. This conclusion is based on their low toxicity to males and females, the absence of negative effects on the aggressiveness of males (based on the high rates of insemination), and on the high degree of infertility of eggs oviposited by treated flies. Although the mode of the action of these compounds is not known, it has been proposed (Jurd et al. 1979) that sterilant activity may be due to microsomal oxidation to biologically active quinone methides.

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SEASONAL OCCURRENCE OF THE PRIMARY AND SECONDARY SCREWORM (DIPTERA: CALLIPHORIDAE) IN THE PACIFIC COASTAL AREA OF CHIAPAS, MEXICO DURING 1978-1979

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Abstract. During 1978 in the Pacific coastal area of Chiapas, Mexico, populations of the primary screwworm, *Cochliomyia hominivorax*, reached seasonal highs in July and November-December and a seasonal low in April. Secondary screwworm, *Cochliomyia macellaria*, population trends were similar, peaking in July and November with a seasonal low in March. Collection of egg masses from sheep showed a 98+% natural viability rate. Data from trap captures and sentinel animals indicate the presence of a resident population sufficient to provide the base for a buildup in screwworm infestations at any time during the year.

In 1972 the United States signed an agreement with Mexico to eradicate the primary screwworm, *Cochliomyia hominivorax* (Coquerel), from most of that country and to establish a new barrier zone across the narrowest part of Mexico at the Isthmus of Tehuantepec. During 1976 a production facility was completed, and fly production was initiated near the city of Tuxtla Gutierrez, Chiapas, which is located in the future barrier zone. While all sterile flies produced in the new factory are currently being released north of the Isthmus of Tehuantepec, progress is being made toward the goal of establishing this new barrier zone. Little is known about the biology and ecology of the screwworm in southern Mexico. The current study was undertaken to obtain information on the seasonal fluctuation of primary screwworm populations in an area (Pacific coastal) where there is a high incidence of this pest. The closely related secondary screwworm fly, *C. macellaria* (F.), was also included in this study, since large numbers of this species were captured in our traps.

MATERIALS AND METHODS

On 14 March 1978 we began the study by placing 50 wind-oriented traps (Broce et al. 1977)

along the Pacific Coast of Mexico (State of Chiapas) from the municipality of Arriaga to the municipality of Pijijiapan (Fig. 1). The area surveyed is approximately 16 km wide and 100 km long with a mountain range on the northeast and the Pacific Ocean on the southwest. During the rainy season the area is covered by lush native vegetation interspersed with improved pastures, while during the dry season most of the area becomes parched and brown. Each survey trap was baited with 1 bottle of swormlure-2 dispensed with a cotton wick (Coppedge et al. 1977). The traps were checked weekly, the flies sorted and counted, and the swormlure-2 replenished as needed. Every 3 weeks the swormlure-2 and cotton wicks were replaced.

On 16 June 1978, a sentinel sheep-pen operation was begun. Five sheep pens, each containing 2-3 sheep, were constructed in the test area for collection of primary screwworm egg masses. Every 10 days, 1 sheep from each pen was wounded to provide a site for egg mass deposition. The wounds, consisting of 5 cm (2 in.) X-shaped cuts through the skin, were cleaned and debrided daily; they were not artificially infested. Egg masses were collected daily (except on Sundays) and incubated at 30 °C to determine the natural fertility rate. Meteorological data were obtained from a weather station operated by the Secretaría de Agricultura y Recursos Hidráulicos in the town of Tonalá, which is located in the test area. The survey was terminated on 15 March 1979.

RESULTS AND DISCUSSION

The biweekly capture rates of primary and secondary screwworm flies with the corresponding rainfall information are shown in Fig. 2. At certain times of the year high winds blew down some of the traps, and at other times high rainfall prevented entrance into areas where the traps were located. As a result, the data plotted are the average number of flies/trap with a range of 23-50 traps/wk (average of 46.6 traps/wk). During the 54-

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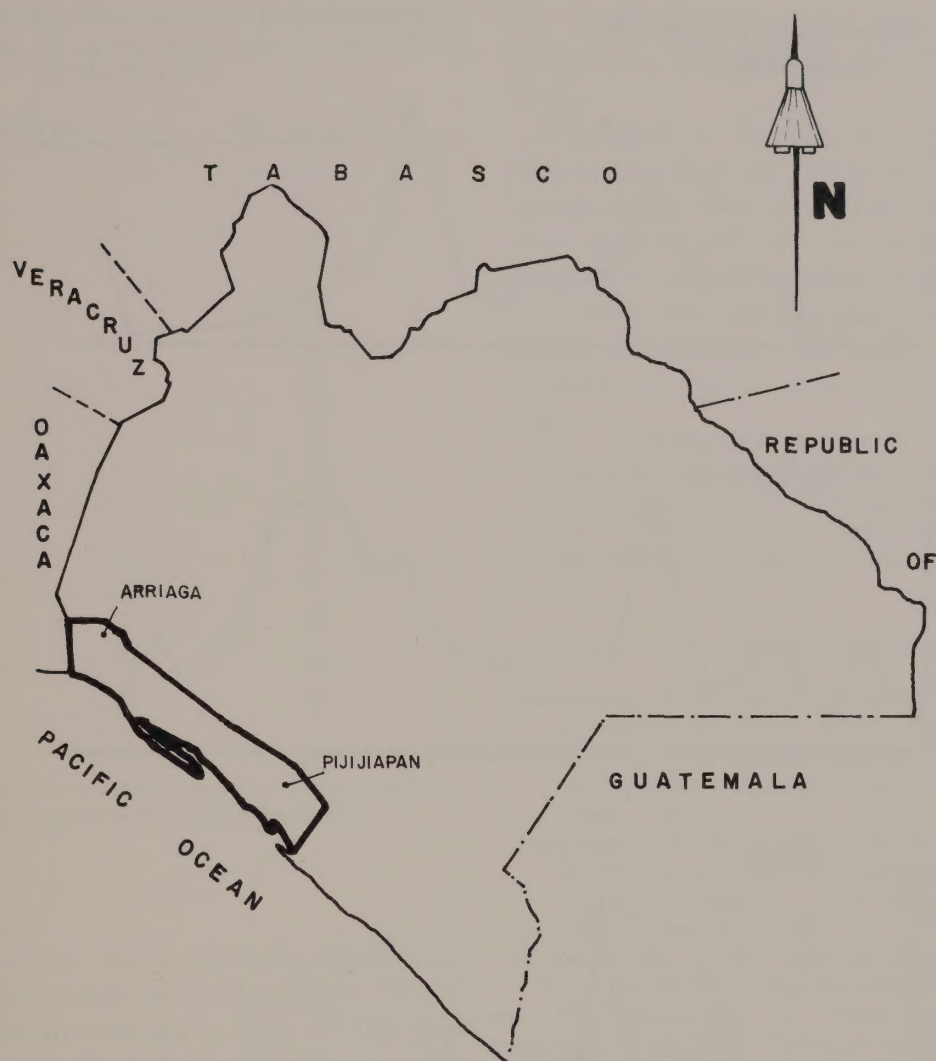


FIG. 1. Map of the state of Chiapas showing experimental area in bold outline.

wk period, a total of 214 male and 1881 female primary screwworm flies were collected. All 50 wind-oriented traps caught primary screwworms during at least part of the survey period. In general the capture rate throughout the year was low, with the lowest periods of collection in April 1978 and March 1979 (0.46 and 0.66 flies/trap/2 wk, respectively). These months are near the end of the dry season and are normally very dry. Baumhover (1963) found screwworm larvae and prepupae to be quite susceptible to desiccation, and the low population during this period can, at least in part, be attributed to this factor. The capture rate of primary screwworms gradually increased from March until it peaked in July at 3.5 flies/trap/2 wk, after which time the rate decreased and remained low for the rest of the rainy season. This sustained reduction in captures probably resulted from a combination of adult inactivity owing to cloudiness and rainfall, and a reduction in emergence because of soil saturation (Parman 1945). It is also possi-

ble that the inclement weather reduced the attractiveness of the swormlure. When rainfall started to decrease the capture rate started to increase, and populations peaked again in November–December. In fact, sustained captures were highest during this period (3.40–3.43 flies/trap/2 wk) when little or no rain fell but soil moisture was still adequate for pupal development and adult emergence. Temperature variations (16.5–23 °C, minimum; 35–41 °C, maximum) throughout the year are so slight that they probably have little effect, if any, on seasonal population trends. In contrast to the seasonal population lows during April 1978 and March 1979 and the seasonal population highs during July and November–December in the tropical area, Jones et al. (1976) reported that the subtropical area around Ciudad Victoria in northern Mexico had a seasonal population low in December and seasonal population highs in August, September, and October. These temporal differences in population highs and lows are probably related to

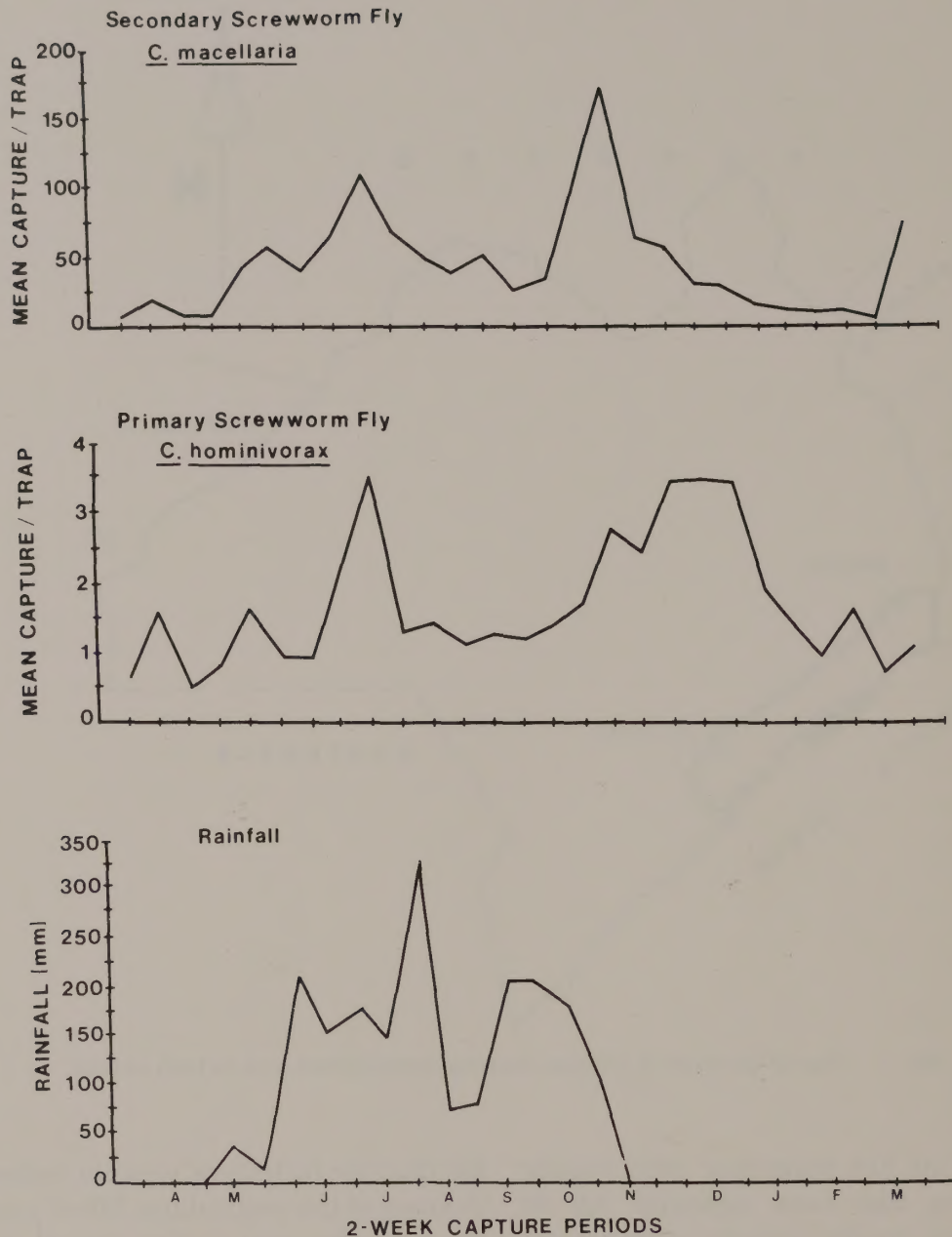


FIG. 2. Rainfall and capture of *C. hominivorax* and *C. macellaria* in swormlure-2 baited traps in a Pacific coastal area of Chiapas, Mexico, 1978–1979.

moisture and temperature differences in the 2 areas.

Screwworm egg mass collection data from sentinel sheep are given in Table 1. In general the collections of egg masses did not correspond well with trap captures of adults. For example, only 12 egg masses were collected in July, a period when trap catches indicated that adult numbers were relatively high, while in August, a period when trap catches indicated adult numbers were low, 40 egg masses were collected. In part these conflicting indications of screwworm activity can be explained by our inability to routinely inspect the sheep during periods of inclement weather; however, there are other considerations. These data may suggest

(as mentioned previously) that screwworm adults may not respond as well to swormlure during wet periods as they do during dry periods. Weather may also affect the attractiveness of a wound on a sentinel animal; during wet intervals a wound stays moist and attractive to screwworms for long periods, while during dry periods a wound desiccates rapidly, thus forming a scab that becomes unattractive to screwworms in only a few hours. Of the 261 egg masses collected 256 hatched, indicating a natural fertility rate of 98.1%.

During the same 54-wk period, 56,249 *C. macellaria* adults were collected, with all 50 traps having positive captures during most time periods. The *C. macellaria* population was always higher than

TABLE 1. Numbers of egg masses collected from sentinel sheep in Arriaga-Pijijiapan during the test period (16 June 1978 to 25 March 1979).

2-WK PERIOD BEGINNING	EGG MASSES*
Jun 16	35
30	21
Jul 14	5
28	7
Aug 11	27
25	13
Sep 8	18
22	14
Oct 6	13
20	7
Nov 17	15
30	12
Dec 1	9
15	14
29	4
Jan 12	8
26	7
Feb 9	8
23	16
Mar 9	8
Total	261

* Of the 261 egg masses collected 256 hatched.

that of *C. hominivorax*, but in general, populations of both species fluctuated at similar times. The *C. macellaria* population reached a high of 105 flies/trap/2 wk in the month of July and another high of 170 flies/trap/2 wk during the month of Novem-

ber. Lows of 4.8 and 4.1 flies/trap/2 wk were recorded for March 1978 and March 1979, respectively.

This study is the first investigation of the population dynamics of the screwworm in the future barrier zone. The data from trap captures and sentinel animals indicate the presence of a resident population whose numbers appear to be correlated to rainfall. The population is present at a level sufficient to provide the nucleus for buildup of screwworm infestations at any time during the year.

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SEPTEMBER 15, 1982

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Results of Analyses
for
U.S. Department of Agriculture

Job No. 15132-682-LA

Table I

Lab Number	Sample Description	sec-Butanol (mg)		Iso-Butanol (mg)	
236524	T-1	Front	<0.002	Front	<0.002
		Back	<0.002	Back	<0.002
		Total	<0.002	Total	<0.002
236525	T-3	Front	0.13	Front	0.083
		Back	<0.002	Back	<0.002
		Total	0.13	Total	0.083
236526	T-5	Front	<0.002	Front	<0.002
		Back	<0.002	Back	<0.002
		Total	<0.002	Total	<0.002
236527	T-7	Front	<0.002	Front	<0.002
		Back	<0.002	Back	<0.002
		Total	<0.002	Total	<0.002
236528	T-19	Front	<0.002	Front	<0.002
		Back	<0.002	Back	<0.002
		Total	<0.002	Total	<0.002
236529	T-21	Front	0.19	Front	0.12
		Back	<0.002	Back	<0.002
		Total	0.19	Total	0.12

Analyses performed by gas chromatography (GC).

Analyses performed in accordance with the NIOSH Method
P&CAM 127.

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Results of Analyses
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Job No. 15132-682-LA

Table II

Lab Number	Sample Description	Dimethyl Disulfide	
		(mg)	
236530	T-2	Front	<0.0005
		Back	<0.0005
		Total	<0.0005
236531	T-4	Front	0.014
		Back	0.0056
		Total	0.020 *
236532	T-6	Front	<0.0005
		Back	<0.0005
		Total	<0.0005
236533	T-8	Front	0.0075
		Back	0.0031
		Total	0.011 *
236534	T-10	Front	0.012
		Back	0.0052
		Total	0.017 *
236535	T-12	Front	0.18
		Back	0.091
		Total	0.27 *
236536	T-14	Front	<0.0005
		Back	<0.0005
		Total	<0.0005
236537	T-16	Front	0.13
		Back	0.068
		Total	0.20 *
236538	T-20	Front	-- **
		Back	-- **
		Total	<0.0005
236539	T-22	Front	0.026
		Back	0.013
		Total	0.039 *

Table II (cont.)

Lab Number	Sample Description	Dimethyl Disulfide	
		(mq)	
236540	T-24	Front	<0.0005
		Back	<0.0005
		Total	<0.0005
236541	T-26	Front	0.16
		Back	0.093
		Total	0.25 *
236542	T-28	Front	<0.0005
		Back	<0.0005
		Total	<0.0005
236543	T-30	Front	0.11
		Back	0.069
		Total	0.18 *

Analysis performed by gas chromatography (GC).

*The analysis of the back sections of the charcoal tubes showed significant breakthrough. NIOSH states that "when the sample value obtained for the backup section of the charcoal tube exceeds 25% of that found on the front section, the possibility of loss exists". During sample storage, the more volatile compounds will migrate throughout the tube until equilibrium is reached (33% of the sample on the backup section).

**The inside of the tube was wet, so both front and back sections were analyzed together.

CLAYTON ENVIRONMENTAL CONSULTANTS, INC.

Results of Analyses
for
U.S. Department of Agriculture

Job No. 15132-682-LA

Table III

<u>Lab Number</u>	<u>Sample Description</u>		<u>Dichlorovos (mg)</u>
236544	T-9	Front	<0.0006
		Back	<0.0006
		Total	<0.0006
236545	T-11	Front	0.098
		Back	0.0012
		Total	0.099
236546	T-13	Front	<0.0006
		Back	<0.0006
		Total	<0.0006
236547	T-15	Front	0.064
		Back	<0.0006
		Total	0.064
236548	T-23	Front	0.0008
		Back	<0.0006
		Total	0.0008
236549	T-25	Front	0.015
		Back	<0.0006
		Total	0.015
236550	T-27	Front	<0.0006
		Back	<0.0006
		Total	<0.0006
236551	T-29	Front	0.0029
		Back	<0.0006
		Total	0.0029

Analytical Method (NIOSH):

P&CAM 295 (GC)

Results have been corrected for a recovery efficiency of >90%.

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Table IV

<u>Lab Number</u>	<u>Sample Description</u>	<u>Particulate (mg)</u>	<u>Indole (mg)</u>
236552	T-17 A-110-37	1.2	<0.001
236553	T-18 A-110-47	1.6	<0.001
236554	T-31 A-110-42	1.0	<0.001
236555	T-32 A-110-39	1.5	<0.001

Analytical Method (NIOSH): S262 (Grav) *

*Analysis performed by gas chromatography (GC).

